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Upper Columbia River region Black bears (*Ursus americanus*) - A DNA mark recapture study

by

Colin Kristian Reynolds

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Biological Sciences

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Dr. Robert Hudson

Dr. John Woods

Dec 17/2001

Abstract

The Upper Columbia River region black bear mark recapture study estimated the black bear population size and density over a 4096 km^2 area of the Upper Columbia River region in British Columbia, Canada, using non-invasive barbwire traps to gather hair roots for genetic tags. The mark recapture estimate utilized a closed mark recapture model that relaxed the assumption of equal effort on every trapping occasion allowing variation in probability of capture among trapping occasions. Mark recapture black bear population size estimate for the Upper Columbia River region is 342 with 95% confidence intervals of 290-421. The density estimate for the Upper Columbia River region study area is therefore 8.3 black bears per 100 km^2 with 95% confidence intervals of 7.1-10.3 black bears per 100 km^2. The estimate of effective population size is 3107 to 15537 black bears. The most significant **r** value from a standardized Mantel test of geographic and genetic distances was found when all the Upper Columbia River region black bears were part of the test.

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To my supervisor and advisory committee, Drs. C. Strobeck, D. Hik, J. Woods and R. Hudson for their advice, and review of this thesis. Maarten van Hof, Gregory Wilson, Chris Kyle and Keri Zitlau for their advice, and review of this thesis. Special thanks to Michael Proctor, Kelly Stalker and David Coltman for their enlightening discussions, Adam Beacham for his friendship and programming of the Mantel test, Kris Reynolds for drawing Figure 3 and to the entire Strobeck lab for putting up with me over the coarse of this project.

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Introduction

How an animal is studied is dictated by the biology of that animal. The biology of black bears, (*Ursus americanus*) increases the difficulty of conducting population scale studies. Biological reasons which contribute to the increased difficulty of studying black bears, particularly on a population level, is that black bears are predominantly solitary (except mothers and cubs), use habitat with thick understory, reside in relatively inaccessible locations and are relatively large animals (adult females 40-70kg, males 60-140kg) which prevents handling without tranquilization (Pelton *et al.* 1999).

These inherent difficulties have resulted in only limited knowledge about the numbers of black bears existing in the wild. Most estimates of black bear populations have not been made using scientific methods, such as mark recapture, that produce population size and density estimates with confidence intervals. The few mark recapture studies done on black bears have been conducted in widely separated parts of the extant black bear range, which extends from Alaska south along the Rocky Mountains into northern Mexico and across Canada into the northeastern United States with a remnant population surviving in the southeastern United States (Bunnell 1987). Mark recapture estimates are available for black bear populations in the southeastern United States (Hellgren & Vaughan 1989; Clark & Smith 1994), the northeastern United States (Garshelis & Visser 1997), eastern Canada (Yodzis & Kolenosky 1986) and Alaska (Miller *et al.* 1997). No mark recapture size and density estimates are available for British Columbia, a part of the core extant black bear range.

The multi-agency West Slopes Bear Research Project (WSBRP) was established to provide information for management and conservation of black bears and brown bears (*U. arctos*) and, as part of this mandate, planned to use mark recapture to estimate the size and density of the black bear population within the

WSBRP's study area (Woods & McLellan 1995). The WSBRP study area was within the Upper Columbia River region, centered on the town of Golden, B.C., and included the eastern portion of Glacier National Park and the western portion of Yoho National Park and considerable non-parklands (Figure 1). Factors affecting the black bear population in the Upper Columbia River region include habitat quality, food resources, hunting, detrimental interactions with human settlements and possible population fragmentation. A size and density estimate of the black bear population in the Upper Columbia River region would be a starting point to understanding the present and long term health of the black bear population in this region.

An estimate of the size and density of a population is a basic requirement in the study of any species and how it fits into its environment. The size and density of the black bear population in the Upper Columbia River region is also critical to wildlife managers. Without this information wildlife managers cannot tell if the black bear population, as a whole, is being affected by human land use (present and future) and by hunting. It will also give the B.C. Ministry of Land, Water and Air Protection a basis on which to judge the health of other black bear populations in the province, with similar habitat and human population, and to estimate other black bear populations for which a mark recapture population estimate or a complete census have not been done.

Mark recapture population estimates have been obtained for black bears by using a number of different tagging methods including ear tags, radio collars, tattoos, tooth dyes and photographs (Yodzis & Kolenosky 1986; Hellgren & Vaughan 1989; Clark & Smith 1994; Garshelis & Visser 1997; Miller *et al.* 1997). Due to the risks to the bears and the high costs, the WSBRP did not want to utilize an invasive method of tagging to obtain mark recapture population estimates and therefore tested two mark-recapture methods (Woods & McLellan 1995). The first relied on remote cameras while the second used genetic tags (Woods *et al.* 1999). The use of genetic tags, for mark recapture of bears, was developed by the WSBRP because molecular methods

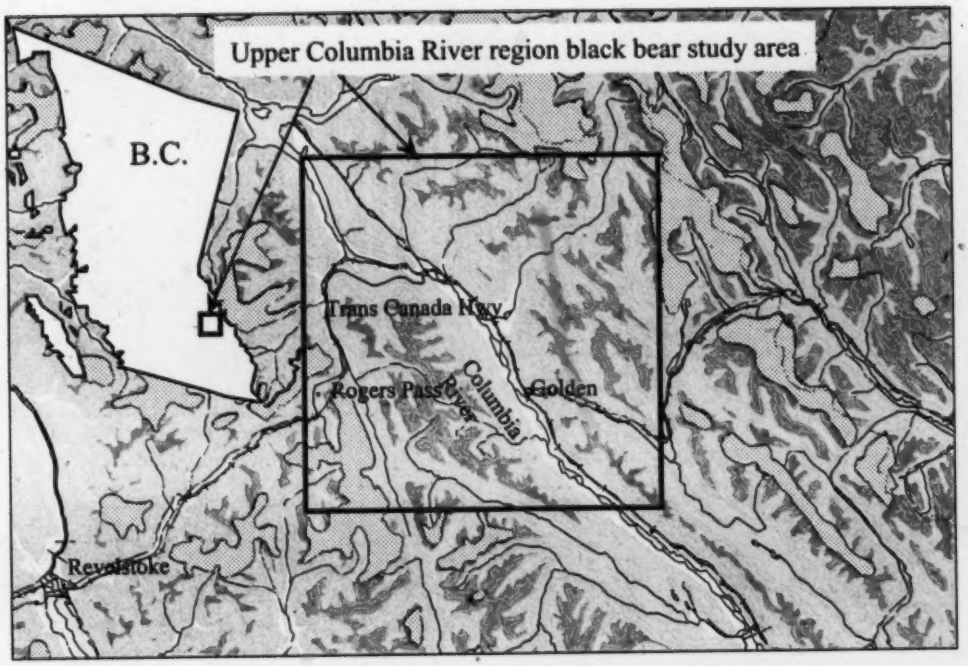

Figure 1 Inset is the province of British Columbia, Canada. The Upper Columbia River region black bear study area is in blue on both the main and inset maps.

had been used to identify individual bears from DNA samples which could be collected without handling a bear (Paetkau & Strobeck 1994, Woods *et al.* 1999).

The use of genetic tags by the WSBRP is a part of the revolution in the field of ecology caused by the adaptation of molecular techniques to address ecological questions (Hughes 1998). Where molecular techniques are practicable they can provide information when sources such as visual identification based on applied tags or morphological characters are unavailable and has greatly increased the ability to recognize individuals (Palsboll *et al.* 1997). The applicability of the genetic tagging method developed by the WSBRP was tested concurrently against remote cameras, these two methods being the most promising methods of data collection for a non-invasive mark recapture study on bears (Woods *et al.* 1999). The field trial demonstrated that the genetic tagging method provided more precise data than could the remote camera method (Woods *et al.* 1999). This superior performance of the genetic tagging methodology compared to the remote cameras led to the adoption of the genetic tagging method for mark recapture population estimates of both black and grizzly bears in the Upper Columbia River region (Woods *et al.* 1999).

The genetic tags used in the mark recapture estimates for the WSBRP utilized microsatelites to identify individual bears. Microsatellites are tandemly repeated DNA sequences from one to six base pairs in length. These sequences are used as molecular markers by designing polymerase chain reaction (PCR) primers for the unique region of DNA, which flanks the microsatellite. The number of repeated sequences within a microsatellite has proved to be highly variable and results in fragment length differences after PCR amplification. The fragment length differences are detected using denaturing polyacrylamide sequencing gels since this method can resolve single base pair differences in DNA.

The combination of PCR and microsatellites has a number of attributes that made this combination a better choice than other molecular methods for the WSBRP as

well as many other projects (Queller *et al.* 1993). The amplification of large amounts of DNA, through the use of PCR, allows non-invasive sampling techniques including hair root removal, as the DNA samples can be of much lower quality and quantity than required for other molecular methods of identifying individuals. PCR primers for nuclear microsatellites amplify fragments for a single locus, with co-dominant alleles that follow standard Mendelian patterns of inheritance. This is a great advantage over multilocus methods, such as DNA fingerprinting with minisatellites where alleles cannot be assigned to specific loci, and methods such as RAPD's and AFLP's with dominant alleles, that have less informative patterns of inheritance. Microsatellites with their high variability and large number of loci present in most genomes (Bruford & Wayne 1993) provide a method of individual identification that can be used even in inbred populations (Menotti-Raymond & O'Brien 1995). Microsatellite genotypes scored with internal standards can be compared between gels allowing large numbers of DNA samples to be examined for individuality and familial relationship. Due to their high level of variability and relatively rapid mutation rate microsatellites are appropriate to study recent evolutionary events, investigate population fragmentation and study populations or species that have undergone bottlenecks.

The non-invasive collection of samples included no visual or handling data on the bears, which meant that the genetic tags had to include more than microsatellite loci. In the Upper Columbia River region black and brown bears are sympatric and because black bears with a cinnamon fur colour are present a molecular method of species identification was required as part of the genetic tag. The molecular identification of species was based on a segment of the mitochondrial control region that was amplified using PCR. The mitochondrial control region was used because of a deletion in this region in brown bears when compared to black bears, which allowed the identification of the bear species of a sample (Paetkau & Strobeck 1996). The use of PCR amplification also made it possible for the genetic tag to include the sex from a

small DNA sample due to the amplification of a section of the Amelogenin gene, which is present on both the X and Y chromosomes and has a deletion in the copy carried on the Y chromosome (Nakahori 1991, Ennis & Gallagher 1994).

Once mark recapture data has been gathered, whether non-invasive or invasive techniques have been used, the first step is to choose the most appropriate mark recapture model with which to make the mark recapture population estimate. The choice of the most appropriate mark recapture model is affected by the biology of the species studied, the study design, the conditions under which the study was conducted, the model estimators which are available and whether the model estimators can handle the type and quantity of data produced by the study. The Upper Columbia River region black bear mark recapture study was designed to allow the use of mark recapture models and estimators for closed populations because a population estimate could be obtained from a single series of trapping occasions over a short period of time. A closed population for a mark recapture study is a population that has undergone no immigration, emigration or mortality during the period of the study (Otis *et al.* 1978). The simplest closed population mark recapture model assumes that no tags are lost, all tags are correctly recorded and that all individuals have equal and constant capture probabilities and that the capture probabilities are not affected by capture (Otis *et al.* 1978).

The two closed population mark recapture models which are most likely to be appropriate for the Upper Columbia River region black bear study relax either the basic assumption that all probabilities of capture are equal or that all individuals have a constant probability of capture. A heterogeneity model which relaxes the assumption of equal probabilities of capture is one of the more appropriate models for a mark recapture estimate of a black bear population because of the biology of black bears. The variation in the size of individual black bear home ranges with the most evident variation being that male black bear home ranges are on average over twice as large as

that of female black bears (WSBRP, unpublished data; Rogers 1987). These differences in home range sizes would result in different levels of opportunity for individual black bears to encounter a trap, which would result in different probabilities of capture. A closed mark recapture model which allows capture probabilities to vary with time is also one of the more appropriate models for a black bear population due to variation in trapping conditions. The attractive power of the baits used for a set of traps could change with weather conditions, availability of food sources and trap topography resulting in varying probabilities of capture on different trapping occasions.

The use of a genetic tag, which includes microsatellite genotypes, as part of a mark recapture study, allows an estimate of the effective population size of the population to be made as well. The effective population size is the number of individuals who contribute genetic information to the next generation (Frankham 1995). An estimate of the effective population size can provide a relative indicator of a population's genetic diversity. As an indicator of genetic diversity the effective population size can be compared with other black bear populations or the Upper Columbia River region black bear population of the future. The effective population size can be estimated for the Upper Columbia River region black bear population because the microsatellite loci used in the Upper Columbia River region black bear study are neutral nuclear markers based on dinucleotide repeats for which there is a range of published mutation rates (Paetkau *et al.* 1998).

The effective population size can highlight situations in which a population is genetically depressed or genetically diverse. A genetically depressed population will have a population size which is larger than it's effective population size and could arise if a population has undergone a population bottleneck from which it has recovered or is undergoing some form of selection which favours the reproduction of only a limited number of individuals (Frankham 1995). A genetically diverse population will have a

population size smaller than the effective population size because the population is mixing or has mixed with surrounding populations (Frankham 1995).

The Upper Columbia River region could have either a genetically depressed or diverse population. The population could be genetically depressed by the selective removal of male black bears of reproductive age or if human land use and/or landscape features has caused the population to be fragmented into non-mixing sub populations. The population could be genetically diverse due to mixing with some or all of the black bear populations that surround the Upper Columbia River region and could reflect a metapopulation of black bears encompassing the regions around the Upper Columbia River region.

The use of genetic tags for the mark recapture population estimate as well as allowing an estimate of the effective population size also allows questions of population structure to be addressed. The first question of population structure to be address by the Upper Columbia River region black bear study is whether the black bears in the region have a genetic relatedness structure reflecting published black bear dispersal patterns. The pattern of dispersal shown by black bears has been males moving large distances and most females staying within or adjacent to their mother's home range (Rogers, 1987). With limited female dispersal a pattern of spatial location and genetic relatedness should be present in which black bears captured physically closer together should be genetically more related than black bears captured farther apart. This pattern should hold for both male and female black bears but female black bears should have a more significant relationship than the entire population with a weaker relationship amongst male black bears than the entire population.

The second question of population structure, which will be addressed, is population fragmentation within the Upper Columbia River region black bear population. The Columbia River and the Trans Canada Highway, which bisect the Upper Columbia River region study area, will be tested as barriers, which have caused

fragmentation of the black bear population in the Upper Columbia River region. The Columbia River and the Trans Canada highway will be tested as barriers which have caused population fragmentation because they bisect the Upper Columbia River region black bear study area and black bears from either side of these landscape features have been treated as a part of a single population for the Upper Columbia River region black bear mark recapture project. The Upper Columbia River region will also be split into four equal quadrants to illustrate the separation caused by geographic distance within the study area, which can be compared to the separation caused by the Columbia River and the Trans Canada highway.

Methods

Study design

The study was designed by the West Slopes Bear Research project and consisted of large and small scale components (Woods & McLellan 1995). The large scale components of the study design are the location of the study, the size, shape of the study, the pattern, number, and density of traps, the number of trapping occasions, and the duration of the study. The small scale components of the study design are the type of trap used and the sites chosen for traps. The study was located in the Upper Columbia River region which includes the city of Golden, B.C. and part of both Yoho and Glacier National Parks and a great deal of provincial land (Figure 1). This location had the advantage of the Rocky and Selkirk mountain ranges to the East and West respectively. The Rocky and Selkirk Mountain divides were hoped to provide a barrier to black bear movement and improve population closure for the black bear population estimate. The Columbia trench region, within which the study was placed, was hoped to contain no barriers to black bear movement allowing all black bears captured in the

study area to be treated as originating from a single population though two possible barriers to black bear movement, the Columbia river and Trans Canada highway, may be creating sub populations.

The study size, 4096 km^2, was chosen to allow the study shape to be square while maintaining the Rocky and Selkirk mountains as the East and West boundaries of the study area. A square shape was chosen to reduce the ratio of edge to area of the study. By reducing the proportion of edge to area of the study it was hoped to reduce the proportion of black bears captured whose home ranges lie partially outside of the study area. Black bears with home ranges primarily outside of the study area by violating the assumption of closure could inflate a mark recapture population estimate if they were captured once and then could not be captured again having moved outside of the study area.

Traps were placed based on a grid pattern that consisted of 64 equal sized cells (Figure 2). The cells were square, 8 km by 8 km (64 km^2). One trap was placed in each cell for each trapping occasion resulting in a trap density of one trap per 64 km^2 which was the highest density of traps possible using the chosen area, the studies budget and having four trapping occasions. The four trapping occasions was a requirement since this was the minimum number of trapping occasions possible with the chosen closed population estimation software (White *et al.* 1982).

The start of trapping for the study was placed after the spring grizzly bear hunting season with the first traps being placed on 9/6/96 (Anonymous, 1996). The end of trapping, 15/7/96, was dictated by the arrival of the berry season and the end of the spring shedding of hair. The berry season reduces the ability of a baited trap to attract black bears and once the black bears have ceased shedding the barbwire used to acquire samples provides mostly broken hairs instead of the required hair roots. With an approximately 6 week window between the end of the grizzly bear hunting season and the start of the berry season/end of the spring shedding of hair with in which to fit

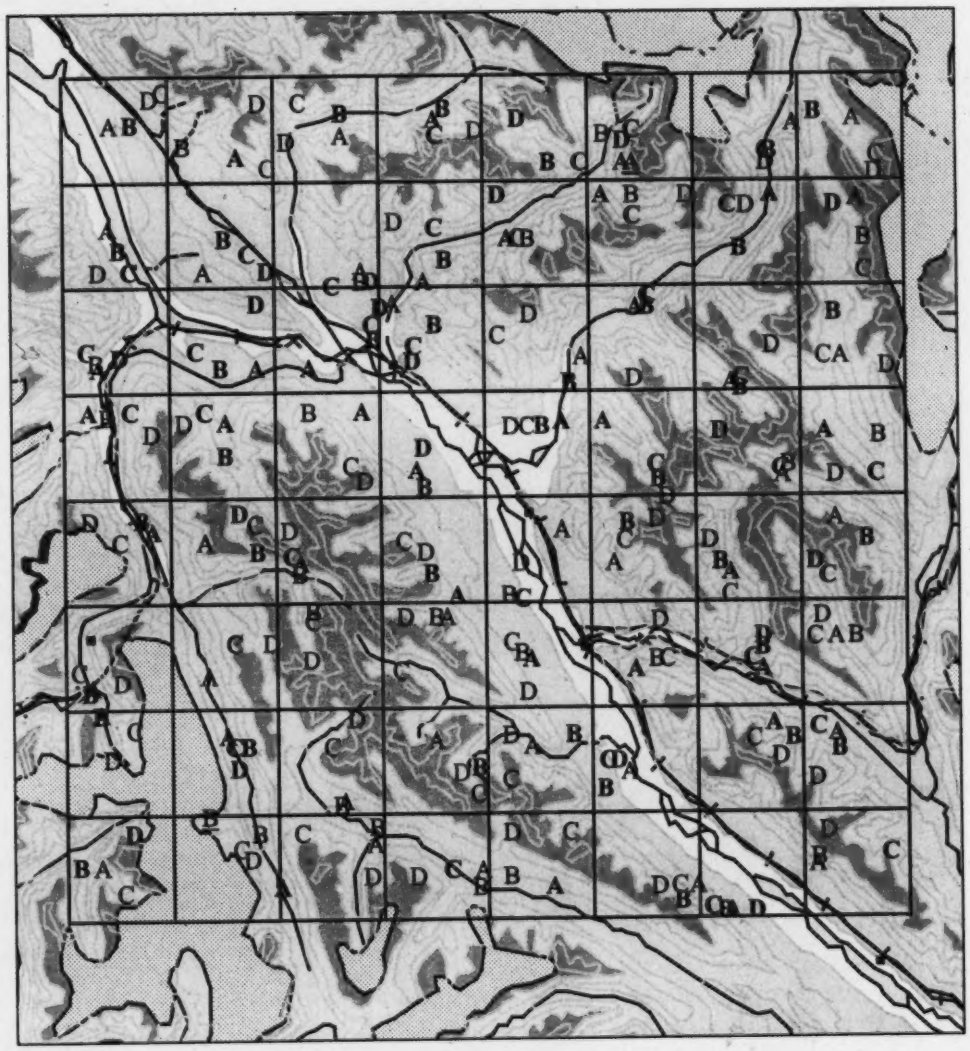

Figure 2 The trapping grid and barb wire trap locations for the Upper Columbia River region black bear mark recapture study. The letters, **A-D**, in bold type are the locations for barb wire traps from trapping occasions A, B, C and D which had black bear hair root samples which produced microsatellite genotypes. If a barbwire trap did not have black bear hair root samples it is shown in normal font. Underlined trap locations were located outside the intended trapping cell.

four trapping occasions, a period of ten days was chosen to leave each trap out before it was moved to a new location. Due to weather conditions and helicopter availability some traps were taken out a day early or a day or two late. When a trapping occasion was finished individual traps were checked for samples, taken apart and moved to a new location. This could not be accomplished for all 64 traps in one day so trap placement was spread over several days. Trapping occasion A, consisted of traps being set out between 9/6/96 and 14/6/96 and then removed between 20/6/96 and 24/6/96 at the same time as the traps for trapping occasion B were setup. The traps for trapping occasion B were removed from 1/7/96 to 4/7/96 and then placed at new locations for trapping occasion C. The traps were again moved between 12/7/96 and 15/7/96 constituting the end of trapping occasion C and the start of trapping occasion D. Trapping occasion D ended between 21/7/96 and 25/7/96.

Barbwire perimeter traps were used to collect all samples. The barbwire perimeter traps consisted of bait hung approximately 4 m above the ground between two trees (Figure 3). The bait was suspended in a burlap sac and consisted of butcher castoffs which had been left outside in a steel drum for at least a month and Alaska fish fertilizer™ applied shortly before the bait was hung. A strand of barbwire was then placed 50 cm off the ground to form a perimeter that the bears would have to cross to investigate the suspended bait. Hairs with roots collected on a single barb were placed in paper envelopes and treated as a single sample. The barbwire was only checked for hair at the end of the trapping occasion at which point the hair was removed and the trap was taken down and moved to a new location where fresh bait was used. The new trapping locations were chosen as locations which the field crew felt were near good grizzly bear habitat while having enough trees to set up a barb wire perimeter trap and could be reached within 30 minutes of hiking from a helicopter landing site.

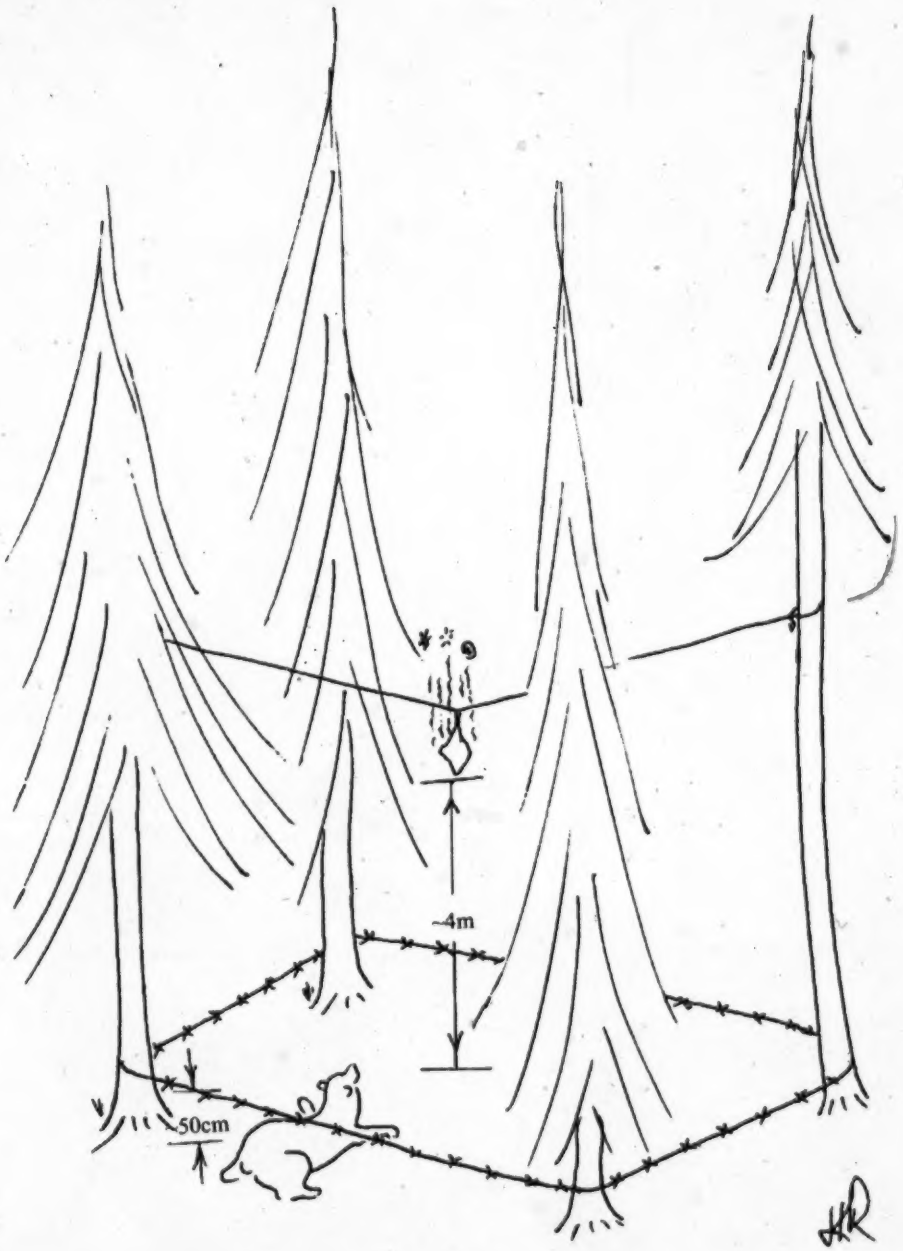

Figure 3 The design of the barb wire traps with suspended bait used for the Upper Columbia River region black bear study.

Samples

Hair samples were placed in labeled paper envelopes in the field and were then stored at -20°C. Each hair sample was examined for hair roots under a dissecting microscope. If hair roots were present, the roots were cut off with about 5 mm of the hair. A maximum of four hair roots were used per sample (or all the roots if less than four were available).

DNA extraction

The DNA was extracted from the hair roots in 200 µL of a 5% Chelex solution (Walsh *et al.* 1991). If the DNA sample was exhausted prior to either a complete microsatellite genotype or the completion of an Amelogenin sex test and at least one hair root was still available the root was then extracted using QIAamp® (maximum of four roots were used).

Species identification

Since grizzly and brown bears are sympatric in the study area and hair colour is highly variable in both species a mitochondria DNA test was used to assign species. The mitochondria DNA test for species is based on a 12-16 bp deletion in the control region of brown bear mitochondria DNA when compared to the control region of black bear mitochondria DNA (Paetkau & Strobeck 1996). The mitochondria DNA control region was amplified using PCR primers and conditions as per Woods et al. (1999). The amplified PCR product was then run on a denaturing polyacrylamide gel, using an ABI 377 DNA sequencer, with known black and brown bear samples as controls.

Samples were then visually scored for species by comparing the samples to the black and brown bear controls using Genescan™.

Individual identification

For samples identified as black bear, a suite of 6 microsatellites (loci G1A, G1D, G10B, G10C, G10L and G10X from Paetkau et al., 1995) was used to determine individual sample identity. Multiplexing by co-amplification was used for five of the six pairs of primers. The two co-amplification groups consisted of the primer pairs for G1D, G10B and G10C (group 1) and G1A and G10L (group 2). One primer from each primer pair of group one was labeled with FAM, group two TET and for G10X HEX. The 30 µl PCR cocktails contained 20 µl of the extracted hair root DNA along with 0.16 µM of each primer, 1.9 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100 and 160 µM of each dNTP and 0.5 U *Taq* DNA polymerase (ddH_2O was used to bring the PCR cocktails to 30 µl). The cycling of the PCR samples was conducted using a Perkin Elmer Cetus 9600 thermal cycler without an oil overlay. The samples were heated to 94 °C for 1 min followed by three cycles of 30 s at 94 °C, 20 s at 54 °C, and 5 s at 72 °C and then 33 cycles of 15 s at 94 °C, 20 s at 54 °C, and 1 s at 72 °C. The cycling was followed by 30 s at 72 °C.

Following PCR, the samples with FAM or TET labeled primers were diluted 1 in 6 into a HEX labeled sample. One gel lane of a denaturing polyacrylamide gel on an ABI 377 DNA sequencer was loaded with 1 µl of mixed PCR product containing the suite of six microsatellite loci along with formamide loading buffer and an internal standard labeled with ROX. The genotypes were scored using Genescan™ and Genotyper™ (Applied Biosystems) and confirmed by independent visual scoring (Figure 4). Any sample with more than two alleles at a locus was classified as mixed and not used in further analysis.

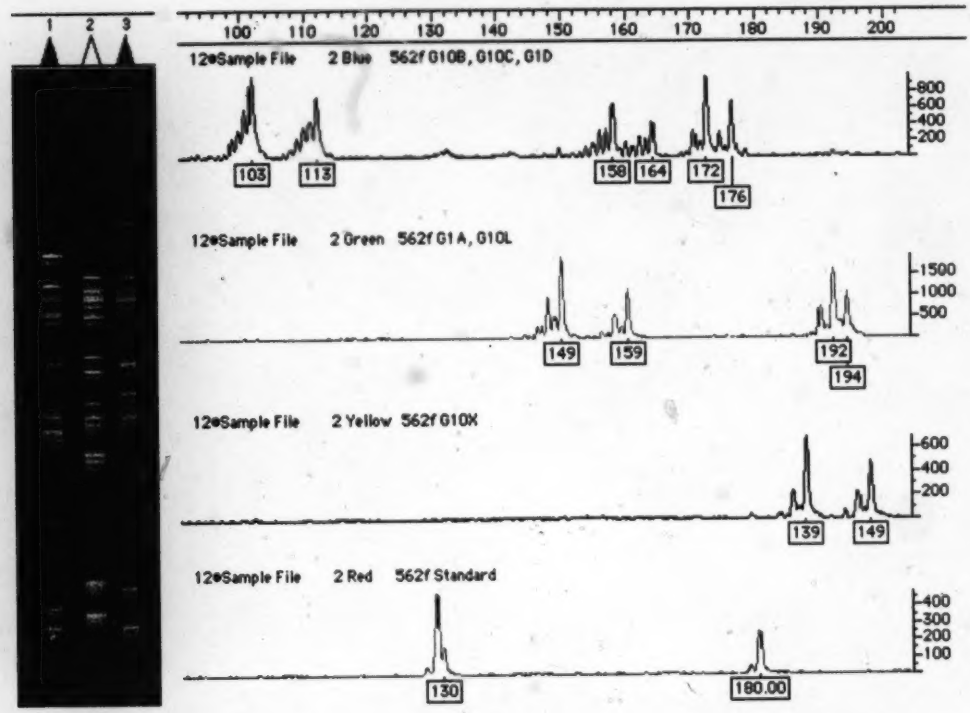

Figure 4 On the left a gel image from an ABI 377 DNA sequencer of three black bear samples amplified using FAM (blue - G1D, G10B and G10C), TET (green - G1A, G10L) and HEX (yellow - G10X) labeled PCR primers and run with a ROX labeled homebrew standard (red). The ABI Genotype™ electrorphenogram of the sample in lane 2 is shown on the right. The alleles observed in the Upper Columbia River region black bear population range for G10C from 99bp to 117bp, G10B from 154bp to 166bp, G1D from 172bp to 190bp, G10L from 135bp to 171bp, G1A from 184bp to 202bp and G10X from 125bp to 163bp.

Genotypes that were miss matches due to a difference of only one or two alleles at one, two or three loci were identified visually or using programs written by the author for Filemaker™. The programs written for Filemaker™ remove both alleles for one, two or three loci in all possible combinations and after each removal searches through all other samples looking for those that match at the loci, which have not been removed. Any samples that matched after the removal of one, two or three loci were double checked for errors in the allele designation and rerun if no error in the allele designation was apparent. None of the full six loci genotypes match using any combination of only three loci.

Samples with the same genotype were accepted as originating from the same individual if there was a less than a 5% chance that this genotype could have occurred in two full siblings of unrelated parents. The chance of full siblings of unrelated parents was used as the basis for accepting samples with the same genotype as coming from the same individual because it would be a more conservative method than one based on random individuals or of a parent and an offspring having the same genotype. To calculate the probability that the genotype at a given locus is the same in two full siblings of unrelated parents is given by the equations

$$P_{sib} = \frac{1 + 2p_i + p_i^2}{4}$$

for a homozygote and

$$P_{sib} = \frac{1 + p_i + p_j + 2p_i p_j}{4}$$

for a heterozygote where p_i is the probability of the ith allele and p_j is the probability of the jth allele (Woods *et al.* 1999). The probability of two full siblings of unrelated parents having the same genotype over any number of loci is the product of each locus.

Once genotypes were accepted as originating from single individuals the microsatellite loci could be tested for Hardy-Weinburg Equilibrium. Each microsatellite locus was tested for Hardy-Weinburg Equilibrium using Genepop version 3.1d (Raymond & Rousset 1995). A significant departure from Hardy-Weinburg Equilibrium may indicate the presence of null alleles, which are alleles which do not amplify because of a difference in the last three bases of the 3' end of either primer, or allelic dropout, which is the result of unequal amplification of one allele in a sample due to the degraded nature of the DNA in a sample or the low concentration of DNA in a sample (Paetkau & Strobeck 1995b, Gagneux *et al.* 1997).

Sex determination

The sex of identified black bear individuals allows population estimates by sex and population structure to be split up by sex, which was required because of the differences in movement of male and female black bears (Rogers 1987). To identify the sex of an individual black bear identified by microsatellite genotype at least one DNA sample that produced the individual black bear microsatellite genotype was sexed using the Amelogenin gene (Nakahori 1991). The Amelogenin gene has deletion in the copy on the Y chromosome compared to the copy on the X chromosome, a feature also present in cattle which enabled the designing of PCR primers that anneal to the flanking sequence of the deletion (Ennis & Gallagher 1994). The PCR primers used to amplify the region of the Amelogenin gene for cattle were used for black bears. The primers used to sex black bears were SE 47 and TET labeled SE 48 (Ennis & Gallagher 1994). The PCR cocktail and amplification conditions used for SE 47 and SE 48 were the same as used for the six microsatellite loci used in this study except that a total volume of 15 µl was used instead of 30 µl. The PCR products were run on 2% agarose gels and/or on an ABI 377 automated DNA sequencer (Figure

Figure 5 A 2% agarose gel stained with ethidium bromide illuminated with UV light. Lanes 1 and 3 are hair root DNA samples from female black bears while lane 2 was a male black bear. All three samples were amplified with PCR primers SE 47 (5'CAGCCAAACCTCCCTCTGC3') and SE 48 (5'CCCGCTTGGTCTTGTCTGTTGC3') (Ennis & Gallagher 1994).

5). Male black bears because of the 30bp deletion in the PCR product from the Amelogenin gene on the Y chromosomes compared to the PCR product from the X chromosomes will have two DNA bands while female black bears will have only one DNA band.

Microsatellite genotype and Sex id consistency check

To confirm the consistency of microsatellite allele designation and sex designation of the black bears identified in the Upper Columbia River region black bear study. The individual black bears were tested for matches with the 127 black bears captured in the Upper Columbia River region prior to the 1996 Upper Columbia River region black bear study (Paetkau D *et al.* 1998). The samples were also checked using the same program written for Filemaker™ by the author as described above which removes one, two or three loci and then checks for matching genotypes.

Population estimation

The Upper Columbia River region study was designed with the intention of using the software program CAPTURE (Otis *et al.* 1978; White *et al.* 1982) to estimate the black bear population. The assumptions, of the mark recapture models and estimators used in CAPTURE, limit the type of mark recapture data appropriate for analysis. One assumption is that traps detain the individuals captured so that captured individuals cannot be captured again during a given trapping occasion. The barbwire perimeter traps used by the study do not detain an individual allowing the possibility of recapture at a different trap during the same trapping occasion. This information was therefore not a part of the mark recapture population estimates but has been included for completeness. A second assumption is that the study area is closed and no

individuals have therefore left or entered the study area during trapping. The Upper Columbia River region black bear study location, size, shape and duration were chosen to help reduce the violation of the assumption of closure. A third assumption is that animals do not lose their marks during the experiment. The choice of a genetic tag was made because this mark cannot be lost by the black bears. A fourth assumption is that all marks are noted and recorded correctly at each sampling occasion which can be met thorough diligent field and laboratory practice. A fifth assumption is that all samples are independent. This would not be the case for a female black bear moving with her cub(s). Therefore, any group of samples that could be parent and offspring (share one allele at all loci), which were found together at more than one trap will be reduced to a single female sample for the mark recapture analysis.

The eight mark recapture models evaluated by the model selection procedure used in CAPTURE are based on the violation of three assumptions. These three assumptions are that there is no variation in individual capture probabilities (heterogeneity), no variation in capture probabilities among trapping occasions (time), and no variation in response to capture (behaviour) (White *et al.* 1982). There are eight mark recapture models which result from not relaxing any of the three assumptions, relaxing one or more of the assumptions and relaxing all three of the assumptions (Table 1). The model selection procedure in CAPTURE uses tests between specific models and general goodness of fit tests on specific models to evaluate a given set of data for the presence of variation in heterogeneity, time and behaviour (Otis *et al.* 1978).

The mark recapture estimate may not be the result of the best model from the model selection procedure because some models do not have associated estimators or the estimators are not appropriate for the Upper Columbia River region black bear data. When CAPTURE was first released it contained mark recapture estimators for five of the eight mark recapture models -M_0, M_h, M_b, M_t and M_{bh} (White *et al.*

Table 1 The models evaluated by the model selection procedure (Otis *et al.* 1978). Models M_b, M_{tb}, M_{th} and M_{bh} have estimators which are inappropriate for use with the type of data collected by the Upper Columbia River region black bear study (see methods) while M_{tbh} has no estimator.

Model	Specific Assumptions
M_0, Constant capture probabilities	No variation in individual capture probabilities, capture probabilities among trapping occasions and in response to capture.
M_h,heterogeneity of individuals	Variation in individual capture probabilities but no variation in capture probabilities among trapping occasions and in response to capture.
M_b, behavioural response	Variation in response to capture but no variation in individual capture probabilities and capture probabilities among trapping occasions.
M_t, variation by time	Variation in capture probabilities among trapping occasions but no variation in individual capture probabilities and in response to capture.
M_{tb}, variation by time and behavioural response	Variation in capture probabilities among trapping occasions and in response to capture but no variation in individual capture probabilities.
M_{th}, variation by time with heterogeneity	Variation in capture probabilities among trapping occasions and individuals but no variation in response to capture.
M_{bh}, behavioural response and heterogeneity	Variation in response to capture and individual capture probabilities but no variation in capture probabilities among trapping occasions.
M_{tbh}, variation by time, behavioural response and heterogeneity	Variation in capture probabilities among trapping occasions, in response to capture and in individual capture probabilities.

1982). Estimators have since been added for M_h, M_t, M_{th} and M_{tb} (Chao 1988, Chao 1989, Chao *et al.* 1992, Stanley &Burnham 1998) but not for M_{tbh}. The estimators for M_h, M_t, M_{th} by Chao are for use with sparse data and will not be appropriate for the Upper Columbia River region black bear data (Chao 1988, Chao 1989, Chao *et al.* 1992). The estimator for M_{tb} (Stanley & Burnham 1998) along with the removal estimators of M_b and M_{bh} (Otis *et al.* 1978) will also not be appropriate for the Upper Columbia River region black bear mark recapture data. The reason the three estimators with a behavioural component will not be appropriate is that the estimators with a behavioural component will fail to produce a good estimate without capture probabilities which are 0.4 or greater on each trapping occasion if four trapping occasions are used (White *et al.* 1982). The population estimate will be the result of the best model chosen by the model selection procedure that has an estimator that can handle the data produced by the Upper Columbia River region black bear study.

There will be four mark recapture population estimates based on four sample groups from the Upper Columbia River region black bear study. The first mark recapture estimate will use all of the sampled black bears and will give the total Upper Columbia River region black bear estimate. The second and third mark recapture population estimates will use only male or female black bear samples respectively, due to the possibility of heterogeneity in the probability of capture between the sexes. The last mark recapture estimate will be a conservative estimate of the number of adult black bears in the Upper Columbia River region, as any possible parent offspring individuals that have been captured at the same trap will be reduced to a single female. This will be a conservative estimate of the number of adult black bears because there is the possibility that adult offspring which move independently may be removed from the analysis as adult offspring have been found with home ranges that overlap that of a parent (Rogers 1987).

Analysis for a trend in behaviour response

The Upper Columbia River region black bear study's use of barb wire perimeter traps was in part because they do not restrain or reward a black bear and should therefore not cause a negative or positive behavioural response in captured black bears. This was a requirement because the intensity of trapping for the Upper Columbia River region, which was feasible, would not be high enough to produce the required capture probability to run estimators for models with a behavioural response component. The selection procedure tests for behavioural response in data but does not indicate if there is a positive or negative trend. To check for possible behavioural trends the percentage of recaptured black bears observed in the Upper Columbia River region was calculated along with, the expected percentage of recaptures if no change in the probability of capture occurred after first capture, if black bears were twice as likely to be captured after first capture, and if black bears were half as likely to be captured after first capture.

The observed percentage of recaptured black bears for a trapping occasion was calculated by dividing the number of observed recaptures by the total number of captures that occurred during that trapping occasion and then multiplying by 100. The expected percentage of recaptures (R) for a given trapping occasion was calculated using:

$$R = \left(\frac{PC}{T}\right)100$$

where P is the probability of capture on a given trapping occasion, C is the number of previously captured bears and T is the number of black bears captured on the same given trapping occasion. To calculate the percentage of expected black bears recaptured if previously captured black bears were either half or twice as likely to be

captured as uncaptured black bears requires the calculation of the probability of capture of previously uncaught individuals. The probability of capture of previously uncaught individuals (p) used was:

$$p = \frac{T}{0.5C + U}$$

if previously captured bears were half as likely to be captured and

$$p = \frac{T}{2C + U}$$

if previously captured bears were twice as likely to be captured where T is the number of black bears captured on a given trapping occasion, C is the number of black bears which have been captured prior to the given trapping occasion and U is the number of black bears which have not been captured (calculated by subtracting the number of previously captured bears from the population estimate). The expected percentage of recaptures (R) for a given trapping occasion when previously captured black bears are half as likely or twice as likely to be captured was then calculated using:

$$R = \left(\frac{0.5pC}{T}\right)100$$

if previously captured bears were half as likely to be captured and

$$R = \left(\frac{2pC}{T}\right)100$$

if previously captured bears were twice as likely to be captured where p is the probability of capture on a given trapping occasion for previously uncaptured black bears, C is the number of previously captured bears and T is the number of black bears captured on the same given trapping occasion.

Behaviour simulations with M_t

The tests for time variation and behaviour variation used in the model selection procedure are sensitive to both types of variation (Otis *et al.* 1978). Since the Upper Columbia River region black bear study will not have the trapping intensity to produce a good population estimate from an estimator for a model which relaxes the assumption of no variation in capture probability after first capture, M_b, M_{bh} and M_{tb} (White *et al.* 1982). The possibility exists of picking a population estimate based on M_t because behavioural variation increased or decreased the number of black bears captured with the same effort in later trapping occasions. Simulations were therefore run using CAPTURE to indicate the extent of behavioural influence for which M_t would be robust. The probability of capture of previously captured individuals was varied from 0.7 to 1.3 of non captured individuals for each of the three trapping occasions (B, C and D) for which there were captured individuals. The time input for the first trapping occasion, T_A, used in the simulations was the observed probability of capture on that trapping occasion divided by the average probability of capture. The time inputs for the last three trapping occasions (T_B, T_C, and T_D) were modified in response to the behavioural inputs in order to keep the combined probability of capture the same as that observed in the Upper Columbia River region black bear data. The time inputs (Ti) for the last three trapping occasions were calculated by dividing the time component of the probability of capture ($\hat{T}_p$) by the average observed probability of capture ($\hat{Ave}_p$):

$$Ti = \frac{\hat{T}_p}{\hat{Ave}_p}$$

where

$$\hat{T}_p = \frac{W}{(XY + Z)}$$

and W is the number of individuals caught on a trapping occasion; X is the behaviour input; Y is the number of previously caught individuals; Z is the number of individuals who have not been previously caught based on the M_t estimate of the Upper Columbia River region black bear data.

Effective population size

The effective population size, which is the size of an idealized population which would give rise to the variance in gene frequency, was calculated as it provides a second measure, along with population size, which can be monitored on an on going basis to gauge the state of a population (Caballero 1994). The effective population size (N_e) was estimated using the equation:

$$H_e = 1 - \left(\frac{1}{\sqrt{1 + 8N_e\mu}} \right)$$

where heterozygosity (He) is averaged for all loci and all individuals, and is based on the stepwise mutation model, and mutation rate μ is in the range of 0.001 to 0.0002 per generation using $(CA)_n$ repeats (Ohta & Kimura 1973; Weber & Wong 1993; Amos *et al.* 1996). With regards to microsatellites the stepwise mutation model would have mutations, which are one repeat larger or smaller than an existing allele and the number of repeats would not be constrained (Ohta & Kimura 1973). Although the stepwise mutation model does not entirely match the mutational characteristics of microsatellites it is the most appropriate simple mutation model (Valdes *et al.* 1993; Weber & Wong 1993). Estimates of N_e were made using both ends of the range of mutation rates (Paetkau *et al.* 1998).

Population structure

The black bear dispersal pattern in which female black bears rarely disperse away from their natal ranges while most male black bears disperse (Rogers 1987) should create a pattern of relatedness within a black bear population in which individuals located closer together are more related than individuals located farther apart. A normalized Mantel test was used to test the null hypothesis that there was no relationship between spatial location and genetic relatedness within the Upper Columbia River region black bear population (Mantel 1967). The normalized Mantel test was done on both sexes together and apart because there could be a more significant relationship between spatial location and genetic relatedness among female black bears than male black bears due to the limited dispersal of female black bears. The normalized Mantel test yields a *r* statistic with:

$$r = \frac{1}{N-1} \sum_{1 \le i < j \le n} \frac{(x_{ij} - \bar{x})}{\sigma_x} \cdot \frac{(y_{ij} - \bar{y})}{\sigma_y}$$

where standard deviations were calculated using:

$$\sigma_x = \sqrt{\frac{1}{N} \sum_{1 \le i < j \le n} (x_{ij} - \bar{x})^2}$$

$$\sigma_y = \sqrt{\frac{1}{N} \sum_{1 \le i < j \le n} (y_{ij} - \bar{y})^2}$$

and the averages were calculated using:

$$\bar{x} = \frac{1}{N} \sum_{1 \le i < j \le n} x_{ij}$$

$$\bar{y} = \frac{1}{N} \sum_{1 \le i < j \le n} y_{ij}$$

where x_{ij} was the pair wise geographic distance, y_{ij} was the pair wise genetic distance and

$$N = \sum_{1 \le i < j \le n} 1 = \binom{n}{2} = \frac{n(n-1)}{2}$$

with the significance of the test *r* statistic was assessed by ranking with the *r* statistics obtained from 10000 permutations of the geographic distance matrix (Mantel 1967).

The geographic distance matrixes were created using the R package (Legendre & Vaudor 1991) to convert latitude and longitude coordinates of individual black bears into pair wise distances in kilometers. A single geographic location was given for each black bear. If a black bear was sampled at more than a single location the center point between the sampled locations was used for the geographic matrix.

Two methods were used to calculate genetic distances. The first method was allele sharing. An allele sharing distance is calculated using:

1 - (number of alleles shared / total possible alleles)

where the number of alleles shared is 0, 1 or 2 for a particular locus and the total possible alleles is two times the number of loci considered (for the Upper Columbia River region black bears, 6 loci, 12 possible alleles). The distance between two individuals will range between 0.083 (one allele shared) and 1 (no shared alleles). The matrices were constructed using the allele sharing distance page at www.biology.ualberta.ca/jbrzusto/sharedst.htlm.

The finite distances generated using allele sharing distances combined with the large number of pair wise comparisons between individuals lead to the use of relatedness values for the genetic distances between individuals. Relatedness values were adopted because a relatedness value is the result of both shared alleles and the frequency of the alleles in the population resulting in a better distance measure and more continuous genetic distances (Queller & Goodnight 1989). The genetic matrixes based on relatedness values were generated using Relatedness 5.06 (Queller & Goodnight 1989).

Black bears captured at the same location could be first order relatives because of limited female dispersal and the possibility of having sampled non-dispersed family groups. This should create a skewed distribution of pair wise relatedness values when compared to pair wise relatedness values of unrelated black bears. The unrelated black bears for comparison where simulated using the same allele frequencies observed in

the Upper Columbia River region at the six microsatellite loci (G10B, G10C, G10L, G10X, G1A and G1D) and the relatedness values were calculated using Relatedness 5.06 (Queller & Goodnight 1989).

To check that the Upper Columbia River region black bears are a single population and not fragmented into two populations, by the Columbia River or the Trans Canada Highway, individuals were tested to see if they were more likely to have originated in the area which they were sampled or on the other side of either the Columbia River or the Trans Canada highway. This was done using the assignment test which assigns an individual to the group in which the individual's genotype is most likely to occur based on the allele frequencies observed in the groups being tested (Paetkau & Strobeck 1995a). If an individual was captured on both sides of one of the proposed population barriers it was placed into both groups for the assignment test. The Upper Columbia River region was also split into four quadrants (North East, North West, South East and South West) of equal size with the black bears sampled in each quadrant being treated as a separate group. The four groups were tested using the assignment test to compare the frequency with which Upper Columbia River region black bears were reassigned back to the quadrant group from which they were sampled to the frequency with which Upper Columbia River region black bears were reassigned back to the side of the Columbia River or the Trans Canada highway from which they were sampled. This should provide a comparison between separation from geographic distance with that caused by the Columbia River and the Trans Canada highway.

Results

Species identification, individual identification and sex identification

From the four trapping occasions in 1996, 1082 samples were identified as black bear. Of these samples 1039 were successfully amplified to produced microsatellite genotypes. Of the 1039 genotyped samples, 120 samples were mixed (had three alleles at one or more loci) and were removed from the analysis. The mixed sampled originated from traps, which had generated a large number of samples, so that the genotypes that produced the mixed samples were already identified and the removal of the mixed samples would not reduce the number of captures. The remaining 919 samples with microsatellite genotypes consisted of 836 typed at all six loci, 51 typed at five loci and 32 typed at four loci. Samples were only left typed at four or five loci if all extracted DNA was used and no more hair roots were available.

The 919 samples had 185 different genotypes. All of the microsatellite genotypes that represented more than one sample had a probability of less than 5% of being two full siblings that have the same genotype and were therefore treated as the same individual (the samples with 5 or 6 loci genotypes had a probability of less than 1% of being full siblings with the same genotype). With an average of 4.97 hair samples having matching microsatellite genotypes only four of the 185 genotypes did not contain at least one sample, which was amplified at all six loci. The four microsatellite genotypes that did not have a sample with six loci all had a least one sample with five successfully amplified loci. The microsatellite loci G1A, G1D, G10B, G10C, G10L and G10X all conformed to Hardy-Weinburg equilibrium when used on the 185 Upper Columbia River region black bears and therefore all six loci were used for individual identification. The heterozygosity and allele frequencies of

the microsatellites used for the Upper Columbia River region black bear study are in Table 2.

Enough DNA remained or could be extracted, to sex 178 individual black bears identified by unique microsatellite genotypes. The PCR amplification of part of the Amalogenin gene identified 90 black bear genotypes as female and 88 black bear genotypes as male.

The 185 black bear genotypes from the 1996 Upper Columbia River region study matched 26 of 127 previously genotyped black bears from the Upper Columbia River region (Paetkau *et al.* 1998). The 26 black bears previously microsatellite genotyped, which had matching genotypes to the 1996 Upper Columbia River region study, had sex identifications in 16 cases. The sex identifications were made when handling the individuals (10) or using ZFX/ZFY SRY (6) (Aasen & Medrano 1990; Taberlet *et al.* 1993). All sixteen sexual identifications were consistent with the ones obtained using the Amelogenin gene on the 1996 Upper Columbia River region black bears.

Population abundance

The data used for the four mark recapture estimates (total sample, male sample, female sample and conservative sample) of the Upper Columbia River region black bear population is in Figure 6. The conservative sample of black bears differs from the total sample by the removal of ten individuals, which were possible offspring (Table 3). The summary of capture histories for each of the four mark recapture estimates are in Table 4 (A-D). The specific tests of assumptions results are in Table 5 for the total population estimate, Table 6 for the male population estimate, Table 7 for the female population estimate and Table 8 for the conservative population estimate. There is no support for M_h being the model by the specific tests of assumptions for any of the four

Table 2 The distribution of alleles observed in the Upper Columbia River region black bear study. There were 185 black bears sampled with a mean number of alleles of 10.67 and a mean heterozygosity of all six microsatellite loci of 0.8033. The probability of two unrelated individuals being the same at all loci is 1 in 17244817 and the probability that a random unrelated individual will be excluded as a potential parent or offspring given no knowledge of other relatives is 0.972843. Het. stands for heterozygosity, # stands for number, all. stands for alleles, All. stands for allele, obs. stands for observed and Freq. stands for frequency.

Locus	Het.	# of all.	All. name	# obs.	Freq. obs.
G1A	0.7536	8	184	55	0.1486
			188	23	0.0622
			190	9	0.0243
			192	111	0.3000
			194	130	0.3514
			196	8	0.0216
			198	33	0.0892
			202	1	0.0027
G1D	0.7666	10	172	64	0.1730
			174	35	0.0946
			176	155	0.4189
			178	40	0.1081
			180	9	0.0243
			182	28	0.0757
			184	10	0.0270
			186	9	0.0243
			188	15	0.0405
			190	5	0.0135
G10L	0.8344	15	135	27	0.0742
			137	40	0.1099
			141	10	0.0275
			145	23	0.0632
			149	54	0.1484
			151	2	0.0055
			153	2	0.0055
			155	12	0.0330
			157	23	0.0632
			159	122	0.3352
			161	6	0.0165
			163	7	0.0192
			165	10	0.0275
			169	25	0.0687
			171	1	0.0027

Locus	Het.	# of all.	All. name	# obs.	Freq. obs.
G10B	0.7963	7	154	2	0.0054
			156	51	0.1378
			158	80	0.2162
			160	79	0.2135
			162	101	0.2730
			164	52	0.1405
			166	5	0.0135
G10C	0.8149	9	99	110	0.2989
			103	68	0.1848
			105	62	0.1685
			107	27	0.0734
			109	11	0.0299
			111	59	0.1603
			113	18	0.0489
			115	12	0.0326
			117	1	0.0027
G10X	0.8543	15	125	11	0.0297
			127	44	0.1189
			129	12	0.0324
			131	1	0.0027
			133	32	0.0865
			139	34	0.0919
			141	1	0.0027
			145	59	0.1595
			147	73	0.1973
			149	83	0.2243
			151	1	0.0027
			153	3	0.0081
			157	1	0.0027
			159	2	0.0054
			163	13	0.0351

Figure 6 Upper Columbia River region trapping occasions, **A-D**, with the genotype identification number of the black bears captured. **Female** black bears are in bold type, male black bears are underlined and individuals whose sex was not determined are in regular font. If a black bear was also captured prior to the 1996 Upper Columbia River region black bear study their genotype identification numbers are in italics.

Table 3 Individuals who were caught at the same trap and share at least one allele per locus (shared alleles shown in bold except where two possible offspring exist in which case italics were used for the second offspring). Parent offspring groups were assigned numbers followed by p for parent and o for offspring. An individual was designated a parent if the individual was female and the other individual was male, if one individual in a female pair had been captured prior to 1996 (individual identifier in italics), if in the case of group 1 one individual shared alleles with both other individuals who did not share an allele at each locus with each other or if two females made up the pair and no other information was available parent and offspring were assigned arbitrarily. F is female, M is male. Row 1 is the North most row of cells. Col. is Column. Column 1 is the West most column of cells. An allele designation of 000 000 indicates that a microsatellite genotype could not be completed for that locus. For the conservative mark recapture estimate the 10 individuals designated as offspring were not included in the estimate.

Possible parent (p) offspring (o) groups	Ind. Id.	Sex	Trap Cell of capture Row	Col.	Locus G1A	Locus G10B	Locus G10C	Locus G1D	Locus G10L	Locus G10X
1p	091	F	1	3	*184* **192**	158 *162*	*109* **111**	*172* **178**	*157* 169	**127** *163*
1o	090	F			**192** 198	162 **162**	107 **111**	176 **178**	**157** 157	**127** 145
1o	135	M			*184* 194	160 *162*	*109* 113	*172* 176	137 *157*	149 *163*
2p	*061*	F	1	7	**192 192**	**162 162**	**103** 105	176 **176**	**135** 149	149 **149**
2o	054	F			**192 192**	**162 162**	**103** 107	174 **176**	**135** 155	133 **149**
3p	016	F	2	4	192 **194**	**162** 164	**105** 113	**176** 182	**157** 159	139 **149**
3o	017	M			190 **194**	156 **162**	103 **105**	**176** 186	153 **157**	147 **149**
4p	088	F	2	4	**192 194**	156 **162**	**099** 105	176 **176**	149 **163**	**133** 145
4o	119	M			**192 194**	**162** 164	**099** 107	174 **176**	137 **163**	**133** 139
5p	171	F	2	6	**190 198**	160 **162**	**103** 105	**178 182**	**137** 145	139 **147**
5o	015	M			**190 198**	158 **162**	099 **103**	**178 182**	**137** 159	149 **147**
6p	*089*	F	3	3	194 **194**	156 **162**	**103 105**	**176** 176	000 000	**133** 157
6o	109	F			192 **194**	158 **162**	**103 105**	**176** 178	137 149	**133** 147
7p	065	F	4	1	**184** 184	158 **160**	**107** 113	174 **176**	**137** 137	147 **149**
7o	068	F			**184** 194	160 **160**	103 **107**	176 **176**	**137** 159	125 **149**
8p	175	F	4	2	**192 194**	**158 160**	099 **115**	**172** 188	149 **157**	**147** 149
8o	080	M			**192 194**	**158 160**	103 **115**	**172** 172	**157** 159	127 **147**
9p	133	F	5	1	194 **194**	**162** 164	099 **111**	**172** 182	137 **149**	127 **147**
9o	024	F			192 **194**	160 **162**	107 **111**	**172** 176	**149** 159	133 **147**

Table 4 Capture history summaries of black bears from the Upper Columbia River region.

Occasion	A	B	C	D
Black bears caught	72	77	61	29
Total black bears caught	72	126	169	185
Black bears newly caught	72	54	43	16

Number of times a black bear was caught	1	2	3	4
Number of black bears	141	35	8	1

A) All captured black bears

Occasion	A	B	C	D
Male Black bears caught	31	38	32	16
Total male Black bears caught	31	58	77	88
Male Black bears newly caught	31	27	19	11

Number of times a male black bear was caught	1	2	3	4
Number of male black bears	64	19	5	0

B) Male captured black bears

Occasion	A	B	C	D
Female black bears caught	40	36	28	11
Total female black bears caught	40	64	87	90
Female black bears newly caught	40	24	23	3

Number of times a female black bear was caught	1	2	3	4
Number of female black bears	70	16	3	1

C) Female captured black bears

Occasion	A	B	C	D
Black bears caught	68	74	58	29
Total black bears caught	68	119	159	175
Black bears newly caught	68	51	40	16

Number of times a black bear was caught	1	2	3	4
Number of black bears	131	35	8	1

D) Conservative number of captured black bears

Table 5 Tests of assumptions for the total Upper Columbia river region black bear data. Test 1 examines the capture frequencies for evidence of variability among individual capture probabilities. Test 2 is for gross behavior effects on capture probabilities. Test 3 is for the variation in average capture probabilities between trapping occasions but is also sensitive to behavioral effects. Test 4 is for heterogeneity and should not reject if variability among individual capture probabilities is the correct assumption. Test 5 is for trap response and should not reject if trap response to capture is the correct assumption. Test 6 is for time variation and should not reject if variation among trapping occasions is the correct assumption. Test 7 is for choosing between the heterogeneity estimator and the general removal model.

Test #	Source of variation tested for	Null hypothesis	Alternative hypothesis	Chi-square value	df	p value	times cap.
1	Heterogeneity	Model M_0 fits the data	Model M_h fits the data	2.769	1	0.09612	
2	Trap response after first capture	Model M_0 fits the data	Model M_b fits the data	14.67	1	0.00013	
3	Time variation in capture probabilities	Model M_0 fits the data	Model M_t fits the data	38.98	3	0.00000	
4	Trap response and/or time variation given heterogeneity	Model M_h fits the data	Model M_h fails to fit the data	28.51	3	0.00001	
4a	same as above	same as above	same as above	15.74 10.71 11.00	3 3 3	0.00129 0.01338 0.01173	1 2 3
5	Heterogeneity and/or time variation given trap response	Model M_b fits the data	Model M_b fails to fit the data	28.54	4	0.00001	
5a	Heterogeneity and/or time variation using first captures only	First capture probabilities are all constant	First capture probabilities vary by time and/or animals	4.843	2	0.08877	
5b	Heterogeneity and/or time variation using recaptures only	Recapture probabilities are all constant	Recapture probabilities vary by time and/or animals	23.69	2	0.00001	
6	Trap response and/or heterogeneity given time variation	Model M_t fits the data	Model M_t fails to fit the data	85.49	71	0.11479	
7	Trap response given heterogeneity	Model M_h fits the data	Model M_{bh} fits the data	22.88	5	0.00036	

Table 6 Tests of assumptions for the male Upper Columbia river region black bear data. Tests 1 - 7 described in the legend of Table 5.

Test #	Source of variation tested for	Null hypothesis	Alternative hypothesis	Chi-square value	df	p value	times cap.
1	Heterogeneity	Model M_0 fits the data	Model M_h fits the data	0.958	1	0.32759	
2	Trap response after first capture	Model M_0 fits the data	Model M_b fits the data	2.100	1	0.14731	
3	Time variation in capture probabilities	Model M_0 fits the data	Model M_t fits the data	14.35	3	0.00246	
4	Trap response and/or time variation given heterogeneity	Model M_h fits the data	Model M_h fails to fit the data	11.14	3	0.01099	
4a	same as above	same as above	same as above	6.625 8.368	3 3	0.08486 0.03898	1 2
5	Heterogeneity and/or time variation given trap response	Model M_b fits the data	Model M_b fails to fit the data	15.29	4	0.00414	
5a	Heterogeneity and/or time variation using first captures only	First capture probabilities are all constant	First capture probabilities vary by time and/or animals	0.893	2	0.63990	
5b	Heterogeneity and/or time variation using recaptures only	Recapture probabilities are all constant	Recapture probabilities vary by time and/or animals	14.40	2	0.00075	
6	Trap response and/or heterogeneity given time variation	Model M_t fits the data	Model M_t fails to fit the data	29.76	30	0.47825	
7	Trap response given heterogeneity	Model M_h fits the data	Model M_{bh} fits the data	10.94	5	0.05257	

Table 7 Tests of assumptions for the female Upper Columbia river region black bear data. Tests 1 - 7 described in the legend of Table 5.

Test #	Source of variation tested for	Null hypothesis	Alternative hypothesis	Chi-square value	df	p value	times cap.
1	Heterogeneity	Model M_0 fits the data	Model M_h fits the data	1.445	1	0.22938	
2	Trap response after first capture	Model M_0 fits the data	Model M_b fits the data	20.48	1	0.00001	
3	Time variation in capture probabilities	Model M_0 fits the data	Model M_t fits the data	30.51	3	0.00000	
4	Trap response and/or time variation given heterogeneity	Model M_h fits the data	Model M_h fails to fit the data	20.98	3	0.00011	
4a	same as above	same as above	same as above	17.20 7.125	3 3	0.00065 0.06802	1 2
5	Heterogeneity and/or time variation given trap response	Model M_b fits the data	Model M_b fails to fit the data	21.89	4	0.00021	
5a	Heterogeneity and/or time variation using first captures only	First capture probabilities are all constant	First capture probabilities vary by time and/or animals	9.105	2	0.01054	
5b	Heterogeneity and/or time variation using recaptures only	Recapture probabilities are all constant	Recapture probabilities vary by time and/or animals	12.78	2	0.00168	
6	Trap response and/or heterogeneity given time variation	Model M_t fits the data	Model M_t fails to fit the data	57.06	39	0.03094	
7	Trap response given heterogeneity	Model M_h fits the data	Model M_{bh} fits the data	20.45	5	0.00103	

Table 8 Tests of assumptions for the conservative Upper Columbia river region black bear data. Tests 1 - 7 described in the legend of Table 5.

Test #	Source of variation tested for	Null hypothesis	Alternative hypothesis	Chi-square value	d.f.	p value	times cap.
1	Heterogeneity	Model M_0 fits the data	Model M_h fits the data	2.250	1	0.13365	
2	Trap response after first capture	Model M_0 fits the data	Model M_b fits the data	11.96	1	0.00055	
3	Time variation in capture probabilities	Model M_0 fits the data	Model M_t fits the data	34.21	3	0.00000	
4	Trap response and/or time variation given heterogeneity	Model M_h fits the data	Model M_h fails to fit the data	25.74	3	0.00001	
4a	same as above	same as above	same as above	13.46 10.71 11.00	3 3 3	0.00375 0.01338 0.01173	1 2 3
5	Heterogeneity and/or time variation given trap response	Model M_b fits the data	Model M_b fails to fit the data	27.55	4	0.00002	
5a	Heterogeneity and/or time variation using first captures only	First capture probabilities are all constant	First capture probabilities vary by time and/or animals	3.729	2	0.15499	
5b	Heterogeneity and/or time variation using recaptures only	Recapture probabilities are all constant	Recapture probabilities vary by time and/or animals	23.82	2	0.00001	
6	Trap response and/or heterogeneity given time variation	Model M_t fits the data	Model M_t fails to fit the data	79.17	67	0.14665	
7	Trap response given heterogeneity	Model M_h fits the data	Model M_{bh} fits the data	20.60	5	0.00097	

sample groups. There is some support for behavioural variation in the total, female and conservative sample groups, but not in the male black bear group, because test 2, from the specific tests of assumptions, rejects that M_0 fits the data in favour of M_b fitting the data. Test 5, from the specific tests of assumptions, for all four sample groups, rejects that M_b fits the data in favour of M_b not fitting the data and therefore does not indicate strong support for M_b. There is however, no evidence for either a positive or negative trend in the behavioural response in any of the four sample groups as shown by Figures 7-10. This is an important result as the estimator for M_t would not be robust to either a positive or negative trend in behavioural response to capture as indicated for all four sample groups in Table 9. The model M_t has been chosen as the model for the mark recapture estimates of all four sample groups as the tests (3 and 6) for time variation in the specific tests of assumptions supported M_t as a model choice.

The mark recapture estimates for the Upper Columbia River region using M_t are 342 with a 95% confidence interval of 290-421 for the total black bear sample group, 151 with a 95% confidence interval of 122-200 for the male black bear sample group, 168 with a 95% confidence interval of 134-230 for the female black bear sample group and 313 with a 95% confidence interval of 266-384 for the conservative black bear sample group. The mark recapture estimates provide density estimates of 8.3 black bears per 100 km^2 with a 95% confidence interval of 7.1-10.3 per 100 km^2 for the total black bear sample group, 3.7 male black bears per 100 km^2 with a 95% confidence interval of 3.0-4.9 per 100 km^2 for the male black bear sample group, 4.1 female black bears per 100 km^2 with a 95% confidence interval of 3.3-5.6 per 100 km^2 for the female sample group and 7.6 black bears per 100 km^2 with a 95% confidence interval of 6.5-9.4 per 100 km^2 for the conservative sample group. Mark recapture estimates using all the estimators in program CAPTURE is provided in Table 10.

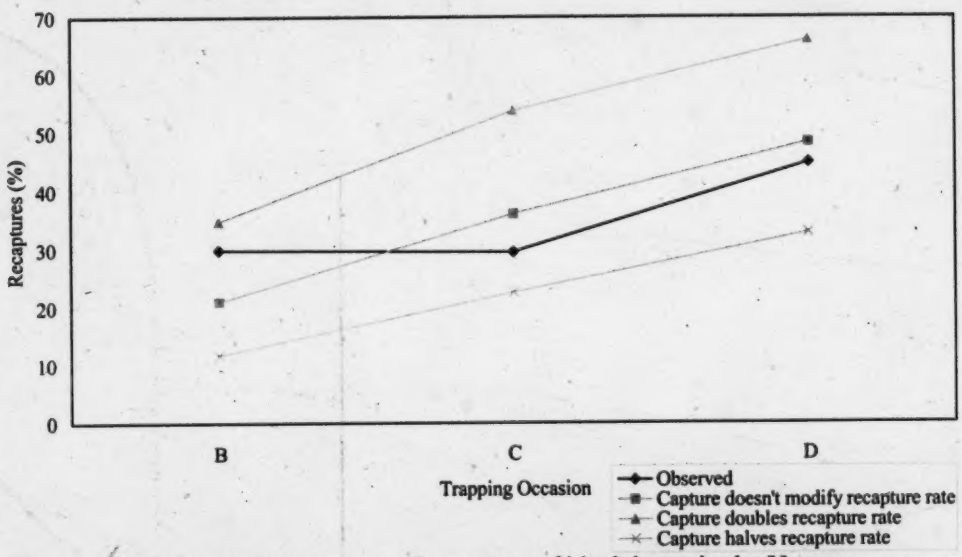

Figure 7 The observed percentage of recapture of black bears in the Upper Columbia River region black bear study with the expected percentage of recaptures if there was no behavioural response to capture and if capture caused a black bear to be either twice as likely or half as likely to be recaptured. Expected recaptures calculated as described in methods.

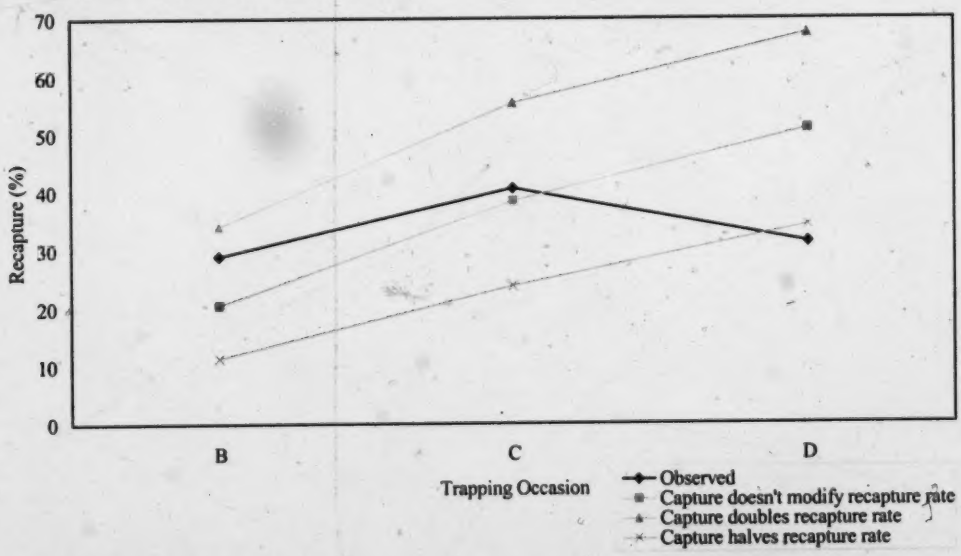

Figure 8 The observed percentage of recapture of male black bears in the Upper Columbia River region black bear study with the expected percentage of recaptures if there was no behavioural response to capture and if capture caused a black bear to be either twice as likely or half as likely to be recaptured. Expected recaptures calculated as described in methods.

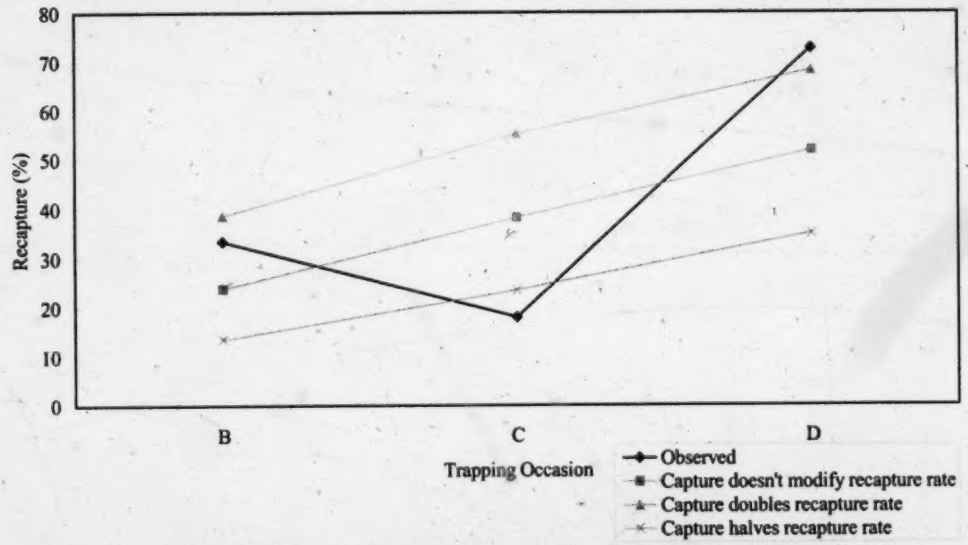

Figure 9 The observed percentage of recapture of female black bears in the Upper Columbia River region black bear study with the expected percentage of recaptures if there was no behavioural response to capture and if capture caused a black bear to be either twice as likely or half as likely to be recaptured. Expected recaptures calculated as described in methods.

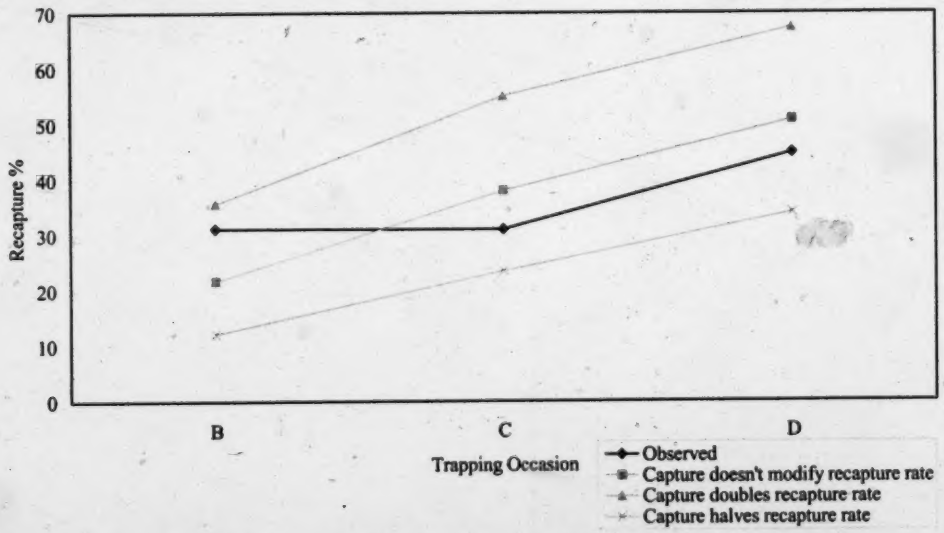

Figure 10 The observed percentage of recapture of the conservative sample group of black bears in the Upper Columbia River region black bear study with the expected percentage of recaptures if there was no behavioural response to capture and if capture caused a black bear to be either twice as likely or half as likely to be recaptured. Expected recaptures calculated as described in methods.

Table 9 Population estimate simulations, using Model M_t, with a behavior response after first capture. Beh. stands for behavioural input which is the change in probability of capture of a previously captured individual from that of an uncaptured individual. The behavioural input of one (in bold) simulates no behavioural response to capture which is the assumption made in M_t. T_A-T_D are the time inputs for trapping occasions A through D. A time input above or below 1 is above or below the mean probability of capture of all four trapping occasions. The time inputs were calculated as described in methods in response to the behavioral input. Het. stands for heterogeneity input which was held constant at the average probability of capture of the four trapping occasions (A-D). M_t ave stands for the average population estimate of 10000 simulations given the behaviour, time and heterogeneity inputs and a population which was actually the one estimated using M_t for the observed data (in bold). % cov stands for percent coverage which is the percentage of estimates which have the true population within the 95% confidence intervals.

Beh	T_A	T_B	T_C	T_D	Het.	M_t ave	% cov
0.7	1.205	1.376	1.148	0.570	.1747	435	42
0.8	1.205	1.345	1.102	0.539	.1747	397	70
0.85	1.205	1.331	1.081	0.524	.1747	382	81
0.9	1.205	1.316	1.060	0.511	.1747	367	90
0.95	1.205	1.302	1.040	0.498	.1747	355	92
1	**1.205**	**1.289**	**1.021**	**0.485**	**.1747**	**343**	**94**
1.05	1.205	1.275	1.002	0.474	.1747	332	94
1.1	1.205	1.262	0.985	0.463	.1747	323	90
1.15	1.205	1.249	0.967	0.452	.1747	314	83
1.2	1.205	1.237	0.951	0.442	.1747	306	72
1.3	1.205	1.212	0.919	0.423	.1747	291	55

Total black bear population

Be.	T_A	T_B	T_C	T_D	Het.	M_t ave	% cov
0.7	1.060	1.384	1.237	0.646	.1937	191	63
0.8	1.060	1.340	1.161	0.592	.1937	175	81
0.85	1.060	1.340	1.161	0.592	.1937	168	86
0.9	1.060	1.326	1.138	0.576	.1937	162	91
0.95	1.060	1.313	1.115	0.561	.1937	156	93
1	**1.060**	**1.299**	**1.094**	**0.547**	**.1937**	**151**	**93**
1.05	1.060	1.286	1.073	0.533	.1937	147	92
1.1	1.060	1.273	1.054	0.521	.1937	143	90
1.15	1.060	1.260	1.034	0.508	.1937	139	88
1.2	1.060	1.248	1.016	0.496	.1937	135	84
1.3	1.060	1.224	0.981	0.474	.1937	129	70

Male black bear population

Beh	T_A	T_B	T_C	T_D	Het.	M_t ave	% cov
0.7	1.391	1.348	1.100	0.453	.1711	215	70
0.8	1.391	1.315	1.054	0.427	.1711	196	84
0.85	1.391	1.299	1.033	0.415	.1711	188	89
0.9	1.391	1.283	1.012	0.404	.1711	181	92
0.95	1.391	1.267	0.993	0.393	.1711	175	94
1	**1.391**	**1.252**	**0.974**	**0.383**	**.1711**	**168**	**95**
1.05	1.391	1.237	0.956	0.373	.1711	163	94
1.1	1.391	1.223	0.938	0.364	.1711	158	92
1.15	1.391	1.209	0.921	0.355	.1711	154	88
1.2	1.391	1.195	0.905	0.347	.1711	150	84
1.3	1.391	1.169	0.874	0.331	.1711	143	74

Female black bear population

Beh	T_A	T_B	T_C	T_D	Het.	M_t ave	% cov
0.7	1.188	1.383	1.144	0.598	.1829	396	42
0.8	1.188	1.351	1.096	0.564	.1829	363	72
0.85	1.188	1.336	1.074	0.548	.1829	348	81
0.9	1.188	1.321	1.053	0.534	.1829	336	88
0.95	1.188	1.307	1.033	0.520	.1829	324	93
1	**1.188**	**1.293**	**1.013**	**0.507**	**.1829**	**314**	**94**
1.05	1.188	1.279	0.994	0.494	.1829	304	94
1.1	1.188	1.265	0.976	0.482	.1829	296	90
1.15	1.188	1.252	0.958	0.471	.1829	288	84
1.2	1.188	1.239	0.942	0.460	.1829	281	75
1.3	1.188	1.213	0.909	0.440	.1829	268	56

Conservative black bear population

Table 10 The mark recapture population estimates supplied by the estimators available in the software package CAPTURE. The estimate using model M_t (in bold) was chosen as the best estimator. M stands for model, Pop est. for population estimate, Std Err for standard error, ci for confidence interval and Den for density.

M	Estimator	Pop est.	Std Err	95% ci	Den /100 km^2	95% ci /100 km^2
M_0	Otis et al, 1978	352	35	297-435	8.6	7.3-10.6
M_h	Otis et al, 1978	375	23	336-425	9.2	8.2-10.4
M_h	Chao 1988	469	70	362-641	11.5	8.8-15.6
M_b	Otis et al, 1978	228	17	206-275	5.6	5.0-6.7
M_t	**Otis et al, 1978**	**342**	**33**	**290-421**	**8.3**	**7.1-10.3**
M_{tb}	Stanley & Burnham 1998	355	493	195-3380	8.7	4.8-82.5
M_{th}	Chao et al, 1992	457	80	340-661	11.2	8.3-16.1
M_{bh}	Otis et al, 1978	228	17	206-275	5.6	5.0-6.7

Total Black bears estimates

M	Estimator	Pop est.	Std Err	95% ci	Den /100 km^2	95% ci /100 km^2
M_0	Otis et al, 1978	154	20	126-206	3.8	3.1-5.0
M_h	Otis et al, 1978	172	15	147-207	4.2	3.6-5.1
M_h	Chao 1988	196	38	144-298	4.8	3.5-7.3
M_b	Otis et al, 1978	118	18	99-175	2.9	2.4-4.3
M_t	**Otis et al, 1978**	**151**	**19**	**122-200**	**3.7**	**3.0-4.9**
M_{tb}	Stanley & Burnham 1998	135	154	91-1106	3.3	2.2-27.0
M_{th}	Chao et al, 1992	189	43	133-313	4.6	3.2-7.6
M_{bh}	Otis et al, 1978	118	18	99-175	2.9	2.4-4.3

Male Black bear estimates

M	Estimator	Pop est.	Std Err	95% ci	Den /100 km^2	95% ci /100 km^2
M_0	Otis et al, 1978	176	26	139-243	4.3	3.4-5.9
M_h	Otis et al, 1978	185	16	159-221	4.5	3.9-5.4
M_h	Chao 1988	243	54	168-390	5.9	4.1-9.5
M_b	Otis et al, 1978	100	6	94-120	2.4	2.3-2.9
M_t	**Otis et al, 1978**	**168**	**24**	**134-230**	**4.1**	**3.3-5.6**
M_{tb}	Stanley & Burnham 1998	1538	20531	106-132512	37.5	2.6-3235.2
M_{th}	Chao et al, 1992	241	63	159-422	5.9	3.9-10.3
M_{bh}	Otis et al, 1978	100	6	94-120	2.4	2.3-2.9

Female Black bear estimates

M	Estimator	Pop est.	Std Err	95% ci	Den /100 km^2	95% ci /100 km^2
M_0	Otis et al, 1978	321	31	272-396	7.8	6.6-9.7
M_h	Otis et al, 1978	350	22	313-398	8.5	7.6-9.7
M_h	Chao 1988	420	61	326-573	10.3	8.0-14.0
M_b	Otis et al, 1978	217	17	195-264	5.3	4.8-6.4
M_t	**Otis et al, 1978**	**313**	**30**	**266-384**	**7.6**	**6.5-9.4**
M_{tb}	Stanley & Burnham 1998	322	493	182-3442	7.9	4.4-84.0
M_{th}	Chao et al, 1992	408	70	306-590	10.0	7.5-14.4
M_{bh}	Otis et al, 1978	217	17	195-264	5.3	4.8-6.4

Conservative Black bear estimates

Effective population size

The average heterozygosity of the six microsatellite loci (G10B, G10C, G10L, G10X, G1A and G1D) of the black bears captured in the Upper Columbia River region black bear study used to calculate the effective population size was 0.80335. The range of mutation rates of 0.001 to 0.0002 per generation for $(CA)_n$ repeats (Amos *et al.* 1996; Weber & Wong 1993) with the average heterozygosity of 0.80335 gives an effective population size range of the black bear population of the Upper Columbia River region of 3107 to 15537 black bears.

Population Structure

The standardized Mantel test **r** values for the pair wise comparison of geographic distance and allele sharing distance is significant for all black bears sampled with **r** = 0.035757 (p = 0.0001) and male black bears with **r** = 0.049538 (p = 0.0005) but is not significant for female black bears with **r** = 0.024297 (p = 0.0657) (Table 11). The standardized Mantel test **r** values for the pair wise comparison of geographic distance and relatedness value (which utilizes allele frequencies which allele sharing does not) had a significant **r** of -0.030290 (p=0.0001) for all black bears sampled. The male black bears **r** = -0.026648 (p=0.0517) and the female black bears **r** = -0.021358 (p=0.0904) were not significant (Table 11). To check for obvious points of leverage which could be causing the significant **r** values the pair wise comparisons between spatial distance and genetic distance were plotted for each sample group and each genetic distance measure (Figures 11-16). Due to the large number of comparisons when the total Upper Columbia River region black bear sample groups spatial and genetic distances are compared the pair wise comparisons have been grouped based on geographic distance between individuals (Figures 17&18). The

Table 11 Standardized Mantel test results using geographic distances and genetic distances (both allele sharing distance and relatedness values). The **r** value produced using a standardized Mantel test is positive when using allele sharing distances due to a higher allele sharing distance for individual pairs who share fewer alleles. The **r** value produced using the standardized Mantel test is negative when using relatedness values due to a lower relatedness value for individual pairs who share fewer alleles. Total is when all 185 black bears sampled were used, female is the 90 female black bears sampled were used and male is the 88 male black bears sampled. The probability, p, of obtaining a higher **r** (allele sharing) or lower r (relatedness) was obtained by randomly permuting the matrixes 10000 times and ranking the observed **r** amongst the **r**'s obtained by permutation. The **r** ave is the average **r** of the 10000 permutations while **r** dev is the standard deviation of the 10000 permutations.

Allele Sharing Distance

Sample	r	p	r ave.	r dev
Total	0.035757	0.0001	0.000091	0.000059
Female	0.024297	0.0657	0.000043	0.000248
Male	0.049538	0.0005	-0.000152	0.000267

Relatedness value

Sample	r	p	r ave	r dev
Total	-0.030290	0.0001	-0.000085	0.000059
Female	-0.021358	0.0904	-0.000034	0.000248
Male	-0.026648	0.0517	-0.000222	0.000267

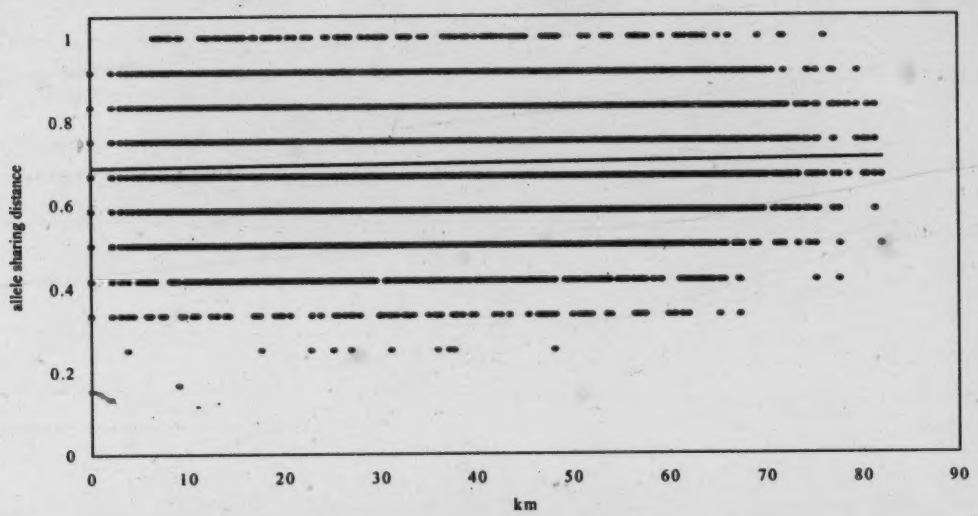

Figure 11 Allele sharing distance and geographic distance pair-wise comparisons among 185 Upper Columbia River region black bears sampled in 1996. The slope of the regression line is 0.000262 allele sharing distance per km.

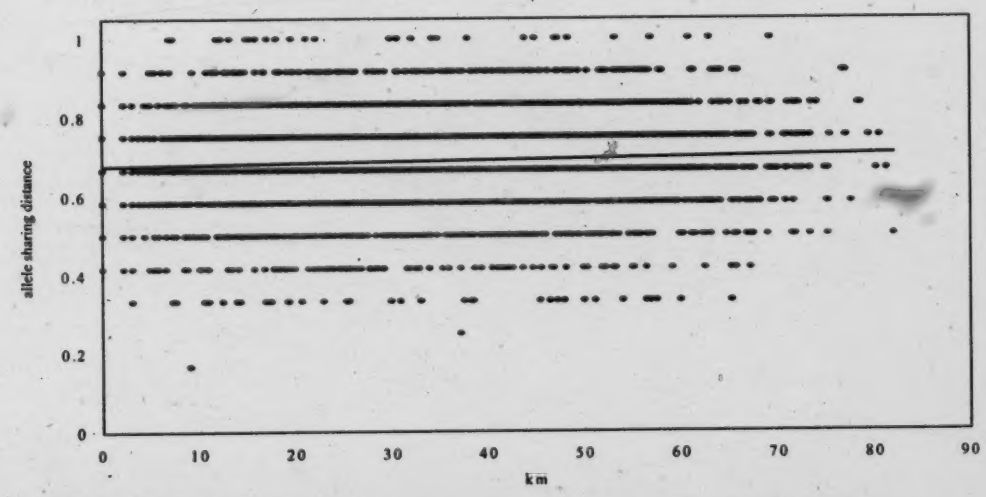

Figure 12 Allele sharing distance and geographic distance pair-wise comparisons among 88 Upper Columbia River region male black bears sampled in 1996. The slope of the regression line is 0.00036 allele sharing distance per km.

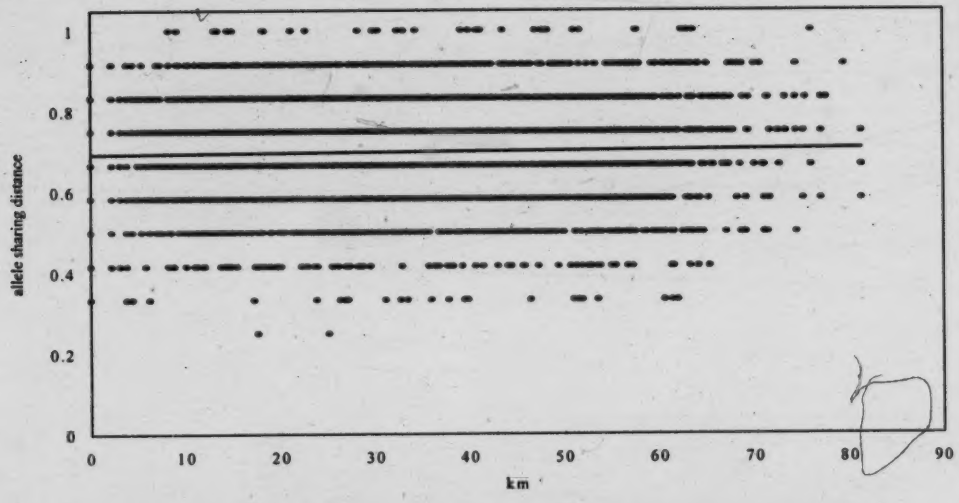

Figure 13 Allele sharing distance and geographic distance pair-wise comparisons among 90 Upper Columbia River region female black bears sampled in 1996. The slope of the regression line is 0.000183 allele sharing distance per km.

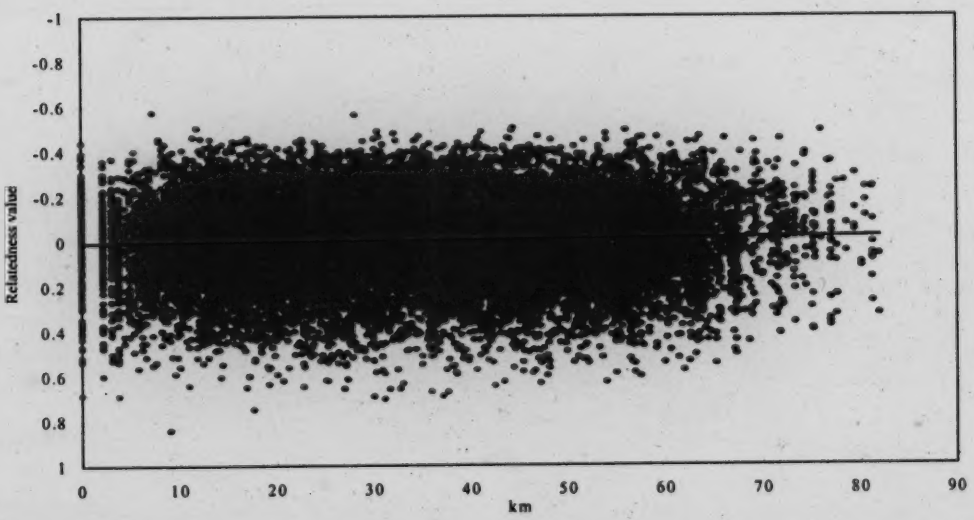

Figure 14 Relatedness value and geographic distance pair-wise comparisons among 185 Upper Columbia River region black bears sampled in 1996. The slope of the regression line is –0.00035 relatedness value per km.

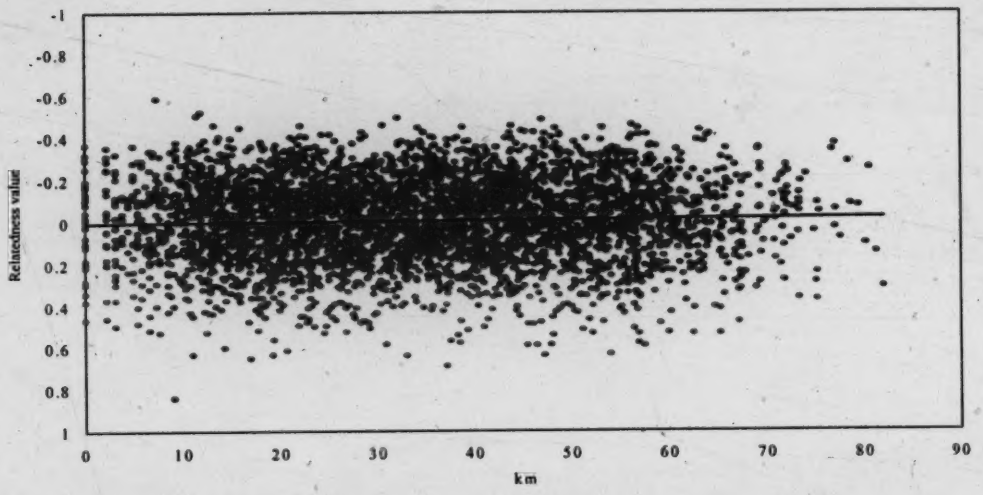

Figure 15 Relatedness value and geographic distance pair-wise comparisons among 88 Upper Columbia River region male black bears sampled in 1996. The slope of the regression line is –0.00031 relatedness value per km.

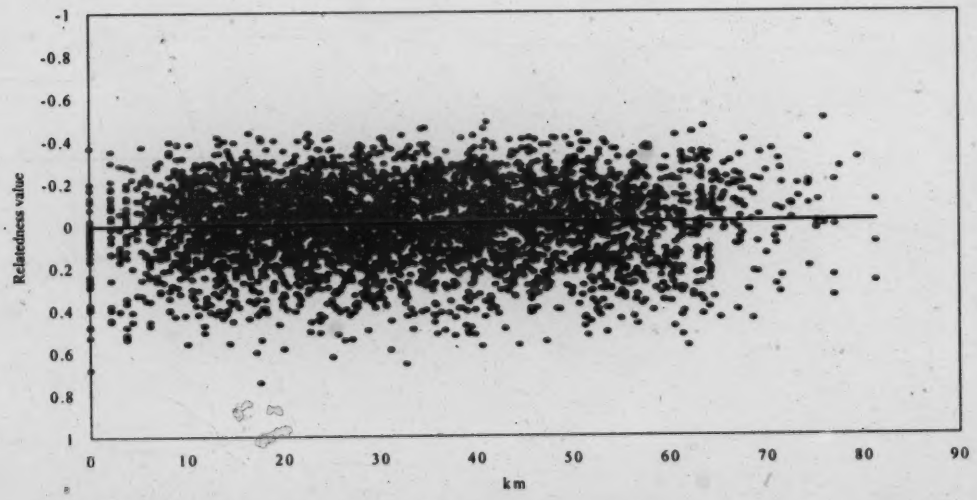

Figure 16 Relatedness value and geographic distance pair-wise comparisons among 90 Upper Columbia River region female black bears sampled in 1996. The slope of the regression line is –0.00025 relatedness value per km.

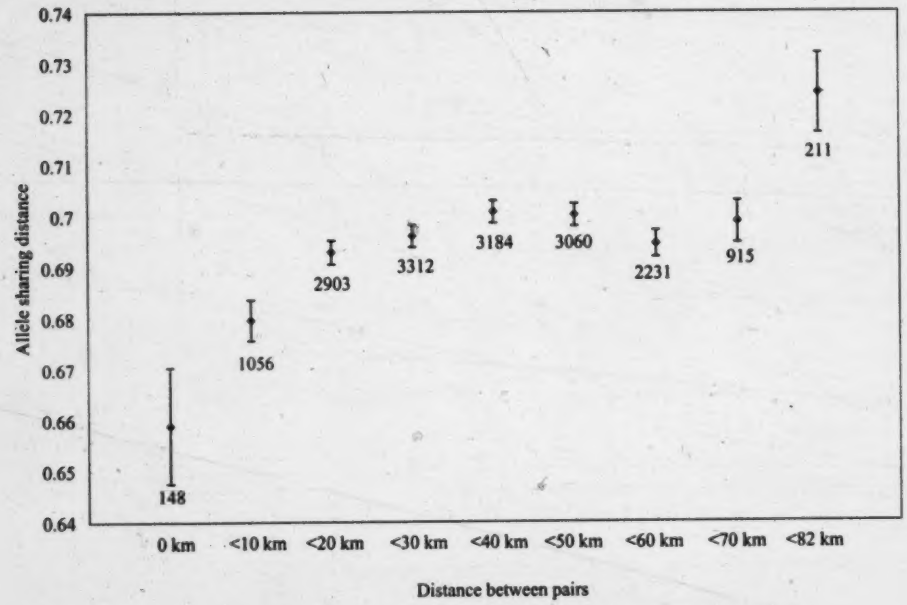

Figure 17 Average allele sharing distance and geographic distance pair-wise comparisons among 185 Upper Columbia River region black bears sampled in 1996.

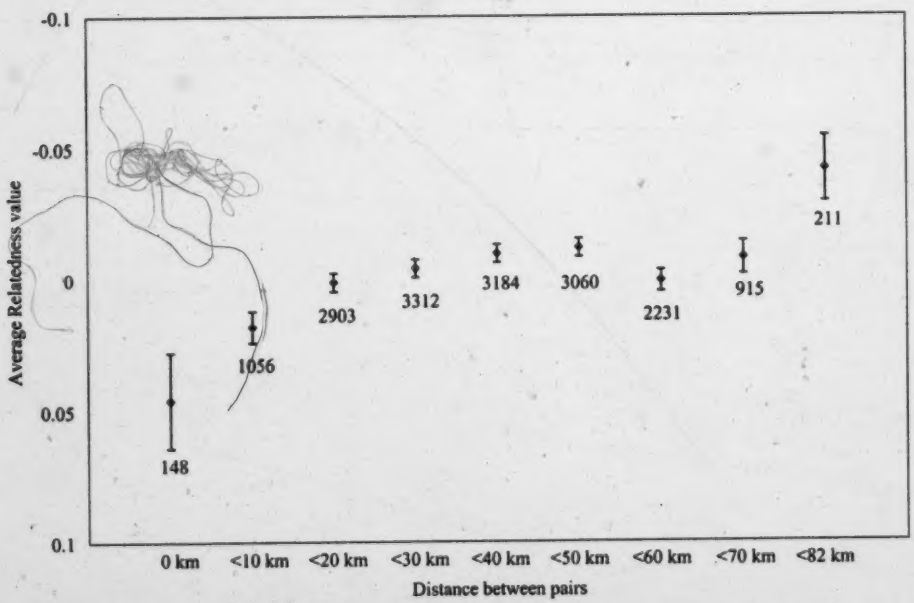

Figure 18 Average relatedness value and geographic distance pair-wise comparisons among 185 Upper Columbia River region black bears sampled in 1996.

distinct drop when black bears were sampled only at the same location could be due to the sampling of possible family groups (Table 3) as well as non first order relatives and would result in a histogram of relatedness values with a bimodal distribution. A histogram of the relatedness values of the samples collected at the same trap were plotted along with simulated unrelated individuals generated from the allele frequencies of the Upper Columbia River region black bears for comparison (Figure 19).

The assignment of Upper Columbia River region black bears on either the East or West side of the Columbia River, using the assignment test (Paetkau & Strobeck 1995a), to demonstrate whether the Columbia River has fragmented the Upper Columbia River region black bear population is in Figure 20 and Table 12a. The results of the assignment of Upper Columbia River region black bears to either the North or South side of the Trans Canada highway using the assignment test (Paetkau & Strobeck 1995a) is in Figure 21 and Table 12b. The results of the assignment of Upper Columbia River region black bears to the four quadrants (North East, North West, South East and South West) of the Upper Columbia River region study area to provide a comparison to the separation of black bears from either side of the Columbia River or the Trans Canada highway is in Table 12d. The capture of individuals in traps on both sides of the Columbia River and Trans Canada highway provides real time evidence of black bears moving across both landscape features. This real time movement and lack of separation of black bears sampled in one area from black bears sampled in another area using the assignment test suggests that the study area does not contain sub-populations of black bears.

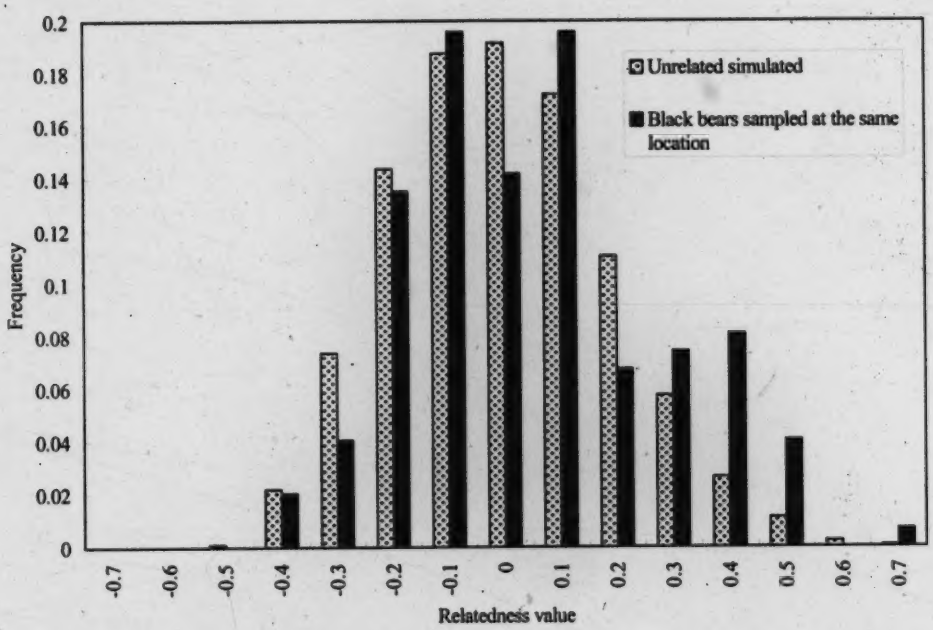

Figure 19 The frequency of relatedness values for the 148 pair wise comparisons between Upper Columbia River region black bears sampled at the same trap (black) and the frequency of unrelated simulated black bears generated from the allele frequencies observed in the Upper Columbia River region (gray).

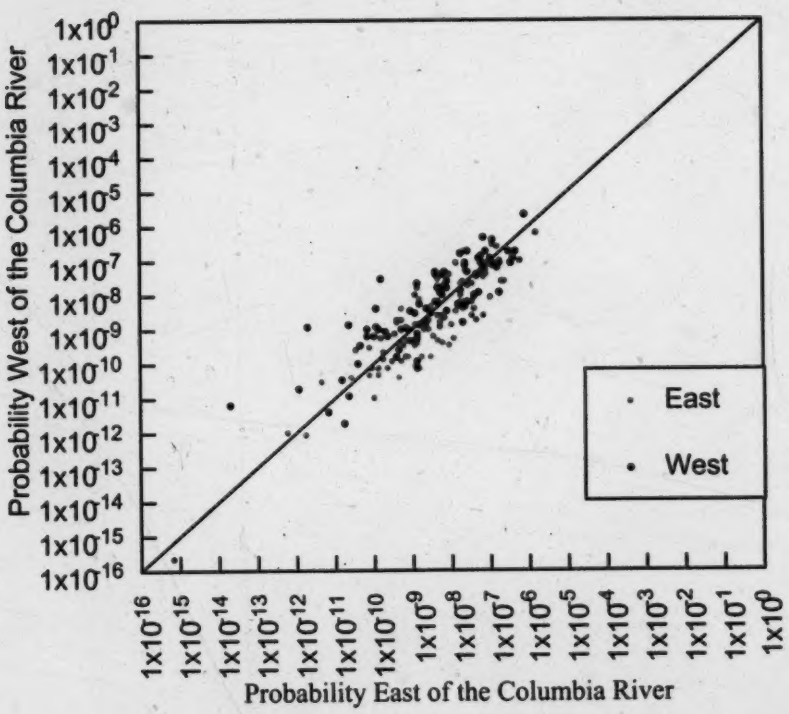

Figure 20 Upper Columbia River region black bears sampled East (gray) and West (black) of the Columbia River. Black bears with genotypes which are more likely to be found in the group sampled East of the Columbia River, based on allele frequencies in both groups, are below the diagonal while those black bears more likely to be sampled West of the Columbia River are above the diagonal.

Table 12 Upper Columbia River region black bear assignment test results (Paetkau & Strobeck 1995a). The assignment test with black bears from West and East of Columbia River had two female black bears (021 & 037) which were sampled both West and East of the Columbia River and were therefore included in both groups. The assignment test with black bears from North and South of the Trans Canada highway had three black bears sampled both North and South of the Trans Canada highway with two being female (037 & 173) and one male (181) all of which were included in both groups. The assignment test which split the Upper Columbia River region black bears into four groups based on the quadrant from which they were sampled had one male black bear (033) sampled in the South West and South East quadrants.

Upper Columbia River region black bears	assigned to the group East of the Columbia River	assigned to the group West of the Columbia River
sampled East of the Columbia River	69	35
sampled West of the Columbia River	33	50

A)

Upper Columbia River region black bears	assigned to the group North of the Trans Canada highway	assigned to the group South of the Trans Canada highway
sampled North of the Trans Canada highway	77	45
sampled South of the Trans Canada highway	23	43

B)

Upper Columbia River region black bears	assigned to the North West quadrant	assigned to the North East quadrant	assigned to the South West quadrant	assigned to the South East quadrant
sampled in the North West quadrant	28	12	14	13
sampled in the North East quadrant	11	20	8	13
sampled in the South West quadrant	4	9	9	11
sampled in the South East quadrant	7	4	10	13

C)

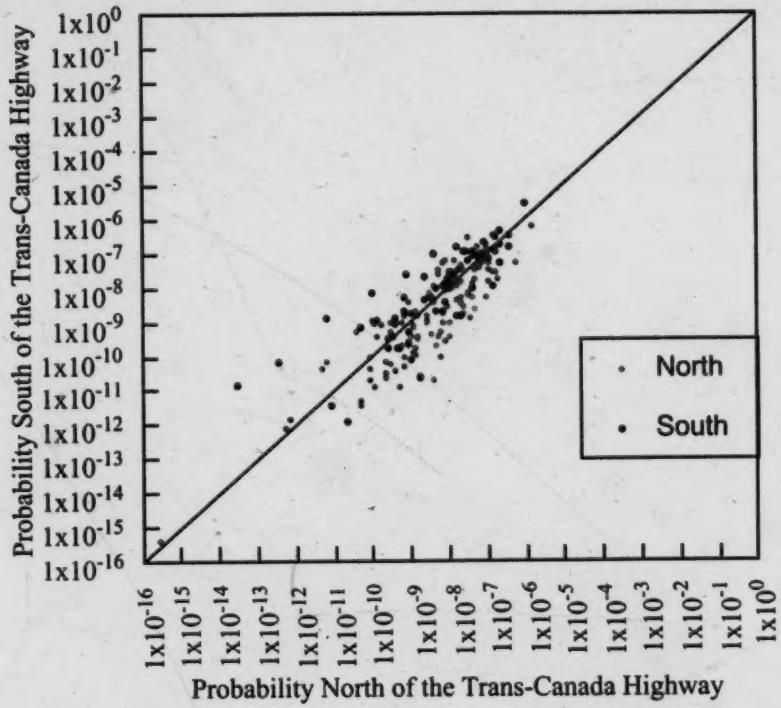

Figure 21 Upper Columbia River region black bears sampled North (black) of the Trans Canada highway and South (gray) of the Trans Canada highway. Black bears with genotypes which are more likely to be found in the group sampled North of the Trans Canada highway, based on the allele frequencies observed in both groups, are below the diagonal while those black bears more likely to be sampled South of the Trans Canada highway are above the diagonal.

Discussion

Study design – area and trap density

The Upper Columbia River region black bear project could have been designed to use either closed or open mark recapture models. A closed mark recapture model assumes that the same individuals present at the start of the study are there at the end while an open mark recapture model will allow migration into or out of the study as well as mortality (Otis *et al.* 1978, Jolly 1965). The Upper Columbia River region black bear project was designed to use closed mark recapture models as these allow a population estimate from a single series of continuous trapping occasions where as the open models assume short trapping occasions with a relatively long period of time between trapping occasions (Otis *et al.* 1978; Jolly 1965). Using an open model for the Upper Columbia River region black bear study would have meant trapping occasions spread over more than a single summer.

The use of a closed mark recapture model to estimate a population's size required the study to be designed to minimize the amount of individual movement in and out of the study area. This is true because this type of movement does not maintain the assumption of closure and can thereby bias the resulting population estimate. Black bear mark recapture studies done on areas less than 500 km^2 have bias because a significant portion of the black bears in the study area will have home ranges that cross the edge of the study due to the relatively large home range size of black bears and will therefore not necessarily be in the study area during all trapping occasions (Garshelis & Visser 1997). The Upper Columbia River region black bear study should not have a significant portion of the black bears with home ranges which cross the edge of the study due to the size, 4096 km^2, and square shape (to minimize

the ratio of edge to area) with the Rocky and Selkirk mountains ranges as the east and west boundaries, respectively.

The compromise made to have the large study size, which would reduce closure bias for the Upper Columbia River region black bear study, was to have a trap density of one trap per 64 km^2. This density resulted in female black bears having 0.91 and male black bears 2.2 traps per mean multi-annual home range. The mean multi-annual home ranges of a female and male black bears in the Upper Columbia River region are 59 km^2, and 138 km^2, respectively (West Slope Bear Research Project, unpublished data). The actual size of a black bear's home range during the 46 days of trapping would however be smaller than the mean multi-annual home range size. The smaller time scale, and thereby reduced home range sizes, would place the traps per individual home range below the 2-4 traps per home range recommended when designing a mark recapture study which will use the closed mark recapture models in CAPTURE to estimate the population size (Otis *et al.* 1978).

Having a trap density below the recommended level should be avoided if possible because this situation, like not minimizing the movement of individuals across a mark recapture study's edge, can cause bias in a closed mark recapture population estimate. The first cause of bias, which mirrors edge effect, is that a trap may or may not be placed in a position where an individual could be captured. As the traps in the Upper Columbia River region black bear study were moved the situation could have occurred in which individual black bears could only be captured on one or a small subset of the trapping occasions. This could produce a situation in which there would be very few individuals captured on multiple occasions. This is reflected in the Upper Columbia River region data as 44 black bears were captured multiple times and 141 black bears were captured only once (Table 4). If individuals are not recaptured due to the movement of a trap outside of their home ranges this can result in a population estimate that is biased high.

The second cause of bias, related to low trapping densities, is that individuals who reside within the study area could have no possibility of being captured. Individuals who had a zero probability of being captured would not contribute to the mark recapture population estimate and would be a factor in creating a population estimate with a low bias. The population estimate made using model M_t, 342 black bears with a 95% confidence interval 290-421, is higher than the number of black bears captured in the Upper Columbia River region black bear study of 1996 by 157 black bears. The addition of 101 black bears that were captured in the two years prior to the Upper Columbia River region black bear mark recapture study, which were not captured during the study, results in 286 total black bears captured. If the mortality rate, which is not known, is ignored the Upper Columbia River region black bear population estimate would be only 56 black bears higher than the total black bears captured in the region while the lower 95% confidence bound is four black bears higher.

Study design – concurrent bear studies

The Upper Columbia River region black bear mark recapture study was conducted at the same time and using the same traps as a mark recapture project on brown bears (Woods et al, 1999). The trouble with doing mark recapture studies on both black and brown bears concurrently arises from the absence of black bears from areas which are not forested, such as alpine regions which contain brown bears, and the absence of brown bears from forested areas with high human activity, which often contain black bears (Pelton et al, 1999, Woods et al, 1999). Within trapping cells, which lack habitat that is utilized by one of the two bear species, trap placement can be based on the best habitat for a particular bear species. In a trapping cell for which a

trap could be placed in habitat in which a black bear or a brown bear would be more likely to be present a decision can be made as to which bear species' capture is preferred.

The priority for the West Slopes Bear Research Project (WSBRP) was the census of brown bears (Woods *et al.* 1999). The emphasis on brown bears resulted in the placement of traps in areas in which the field crew felt would be more likely to catch brown bears. This being the first mark recapture project on brown bears using barbwire traps the knowledge gained from the earlier occasions were utilized in the trap placement in later trapping occasions. In the first two trapping occasions there were fewer brown bears captured, 15 and 16 respectively, and lower average trap elevations, 1,244 m and 1,336 m respectively (Woods *et al.* 1999, West Slopes Bear research project unpublished data). In the last two trapping occasions the number of brown bears captured increased to 22 and 20 respectively while the average trap elevation increased to 1,501 m and 1,626 m respectively (Woods *et al.* 1999, West Slopes Bear research project unpublished data). Over the same four trapping occasions the number of black bears captured went from 72 to 77 to 61 and finally to 29. The increased trap elevation reflects the rising snow line while the different capture rates most likely reflect different habitat usage by the two bear species. Black bears were found to be more tolerant to the transportation corridors in the Upper Columbia River region and were using timbered areas and rights-of-way located in valley bottoms (Munro, 1999). Brown bears were avoiding the transportation corridors and using slide chutes, which are at higher elevations (Munro, 1999).

The change in trap placement during the Upper Columbia River region black bear mark recapture study would result in a situation in which each trapping occasion was not conducted with the same effort with respect to the capturing of black bears. The closed mark recapture model, which relaxes the assumption of equal probability of capture on each trapping occasion, is model M_t (Table 1). M_t, as the model that would

be predicted based on changing trapping effort and is also the model chosen based on the tests of assumptions.

Population estimation – model selection

The model supported by the model selection procedure, M_t, allows variation in the probability of capture between trapping occasions (Tables 5-8). M_t is not robust to directional behavioural variation (animals being trap shy or trap happy, Table 9). Directional behaviour variation does not appear to be present in the data (Figures 7-10) supporting the use of M_t. M_t is not the most biologically appropriate, of the closed mark recapture models, as it does not relax the assumption that all animals have the same probability of capture on a give trapping occasion. Allowing individuals to have different probabilities of capture would be the most biologically appropriate due to the placement of bears on the landscape being such that they all would not all be the same distance from the closest trap and would therefore have a different chances of being captured. Model M_h, which allows heterogeneity of individual capture probabilities, was not used for the Upper Columbia River region black bear population size estimate, as there was no support for M_h in the tests of assumptions. The support in favour of M_t, instead of M_h, is most likely due to the magnitude of the violation of the assumption of equal effort on each trapping occasion, which resulted in the differences in the number of black bears captured on each trapping occasion (Table 4).

Model M_b was not supported by the tests of assumptions and there was no evidence of a directional behavioural response to capture (Tables 5-8, Figures 7-10). These are critical result for two reasons. The first being that with the low trap density and thereby low rate of capture for the Upper Columbia River region black bear study a model that relaxes the assumption of no change of capture probability after first capture could not be used. A probability of capture of at least 0.4 per trapping

occasion is required when using four trapping occasions or the estimators associated with models which allow behaviour variation will fail (White *et al.* 1982). The Upper Columbia River region black bear study's average probability of capture per trapping occasion, based on the total population estimate using M_t, was 0.175. With an average probability of capture per trapping occasion less than 0.4 the models that relax the assumption of no behaviour variation produced estimates below the 286 black bears captured in the Upper Columbia River region from 1994-1996 in the case of M_b and M_{bh} and an estimate with a standard error larger than the estimate along with 95% confidence interval that extends an order of magnitude above the estimate in the case of M_{tb} (Table 10). The second reason the absence of behavioural variation is a critical result is that estimates based on M_t are not robust to directional behaviour variation (Table 9). The lack of behavioural response to trapping is a support for the use of barbwire traps as they were hoped not to produce a behavioural response. The lack of positive response was hoped to occur because the baits were hung out of reach of the bears while a negative response was avoided by not having to handle or restrain the bears in anyway.

Population estimation – sample groups

The black bears captured during the Upper Columbia River region black bear mark recapture project were divided into four different sample groups for four different population estimates. One sample group contained all the black bears sampled during the Upper Columbia River region mark recapture study and this group was used for the total Upper Columbia River region black bear population estimate. No black bears were excluded from this estimate because there was no conclusive evidence for violation of the assumption that individuals move independently which is part of closed mark recapture models (Otis *et al.* 1978). Individuals would have been

excluded for violating the assumption of independence if they had possible parent offspring microsatellite genotypes (at least one matching allele at all loci) and were captured together at two or more traps. The assumption of independent movement may be violated in the case of ten individuals who have possible parent offspring microsatellite genotypes with a female black bear but were captured together at a single trap.

The possibility of individuals violating the assumption of independence of movement warranted a conservative population estimate of the Upper Columbia River region. This estimate is based on all of the black bears captured in the Upper Columbia River region mark recapture project except ten individuals who were possible offspring in family groups (Table 3). The conservative population estimate, 313 black bears, is an estimate of the minimum number of sub-adult and adult black bears (Table 10). This is a minimum estimate as a portion of the ten individuals removed may be adult offspring who move independently. Sharing an allele at all loci and being captured by a single trap is not conclusive evidence of being part of a family group because female black bears setup home ranges within or adjacent to that of their mother's home range more often than they disperse (Rogers 1987). The possibility then exists of an adult female black bear and her mother and/or father having home ranges such that they are attracted to and sampled at the same barbwire trap. This would produce individuals captured at the same trap who have microsatellite genotypes indicating that they could be parent and offspring but not be in violation of the closed mark recapture model assumption of independent of movement.

The other two sample groups from the Upper Columbia River region mark recapture study are male and female black bears separately. This was done due to the possibility of heterogeneity in capture rates between the two sexes, which could most likely be the result of the difference in home range size between the sexes. The mark recapture population estimate of the Upper Columbia River region male black bears is

89.9% of the female black bear estimate. A higher female black bear population is expected as female black bears with cubs are protected from hunting (Anonymous 1996). The number of individual male black bears captured was however, 97.8% of the number of individual female black bears captured. The proportionally larger female black bear estimate, on approximately the same number of individuals captured, could also be the result the lower proportion of female black bears recaptures (21.7%) versus male black bear recaptures (24.8%). The cause of the lower proportion of recaptures in female black bears in the Columbia River region maybe the result of smaller home range size of female black bears. The smaller home range size would result in a lower chance of a trap being within a female black bear's home range on multiple trapping occasions.

The Upper Columbia Region estimate from combining the male and female estimates is lower than the total estimate. This difference is most likely the result of seven black bears that were not included in either the male or the female estimates as there were no sex identifications for these black bears because there was no more DNA or hair root available. The estimate from combining male and female estimates is still larger than the estimate from the conservative sample group, which had three fewer black bears than the combined male and female sample groups.

Population estimate – density comparison

The total population density estimate for the Upper Columbia River region is amongst the lower black bear density estimates (Table 13). The closest density estimates to the Upper Columbia River region estimate of 8.3 black bears per 100 km^2 are from two black bear populations in Arkansas, with 7.5 and 9 black bears per 100 km^2 and one black bear population in interior Alaska with 8.9 black bears per 100 km^2 (Clark Smith 1994, Miller *et al.* 1997). The density estimate from Alaska did not include areas above 1,524 m while the Upper Columbia River region estimate includes

Table 13 Black bear densities estimated using mark recapture techniques.

Location	Black bears per 100 km^2	Estimate year	Reference
White Rock Arkansas	7.5	1989	Clark Smith 1994
Middle Susitna River Basin Alaska	8.9	1985	Miller *et al.* 1997
Dry Creek Arkansas	9.0	1989	Clark Smith 1994
Michigan	15.1	1991	Garshelis &Visser 1997
Minnesota	18.8	1991	Garshelis &Visser 1997
Kenai Peninsula 1947 burn Alaska	19.9	1986	Miller *et al.* 1997
Kenai Peninsula 1967 burn Alaska	28.9	1986	Miller *et al.* 1997
East central Ontario	50.1	1977	Yodzis & Kolenosky 1986
Eastern Virgina and northeastern North Carolina	52-66	1984-1986	Hellgren & Vaughan 1989

all elevations though in five cells in the Purcell Mountains no black bears were captured (Figure 6). If these cells are not included the density estimate increases to 9.1 black bears per 100 km^2, which still places the estimate of the Upper Columbia River region in the low end of black bear density estimates.

The populations with low density estimates, though in very separate parts of North America, have the commonalities of being harvested populations which do not have access to fish runs.

Effective population size

The effective population size is much higher than the estimated population size of 342 black bears thereby indicating a genetically diverse population (Ohta & Kimura 1973). The insular black bear population in Newfoundland in comparison has an effective population size estimate of 239 to 1195 with an estimated population size of 3000 to 10,000 (Paetkau *et al.* 1998). The Upper Columbia River region black bear populations genetic diversity could not be maintained in isolation so the population is either not isolated or has just recently become so which is not likely.

Population structure – mantel test

Black bear dispersal patterns have been observed such that male black bear offspring always disperse while most females black bears setup home ranges within or adjacent to their natal home range (Rogers 1987). This is expected to setup a population structure in which black bears sampled closer together are more genetically related than animals sampled farther apart. This pattern of population structure is expected to be more apparent among female black bears than among male black bears. This should result in r-values when doing a standardized Mantel test that are more

significant for female black bears than male black bears. When the sexes are combined the level of significance should be between that of female black bears and male black bears.

This pattern was not seen in the Upper Columbia River region black bear population. The most significant r-value using both allele sharing and relatedness value was with the sexes combined. This result is probably due to the presence of family groups, sampled together, as there is a bi modal distribution of relatedness values seen in the Upper Columbia River region black bears sampled at the same trap (Figure 19). The simulated black bears with the same allele frequencies as found in the Upper Columbia River region black bear population does not have a bi-modal distribution (Figure 19).

Population structure – assignment test

The Upper Columbia River region was believed to be an area with a black bear population that was not fragmented. Two possible barriers to black bear movement tested using the assignment test were the Columbia River and Trans-Canada highway. Neither the Columbia River nor the Trans-Canada highway appear to be fragmenting the Upper Columbia River region black bear population as 36% (in both cases) of black bears had allele frequencies which were more common on the other side of the possible barrier than from the side on which they were sampled (Table 12). In the case of the Columbia River there were also 2 black bears sampled at traps on both sides of the river while 3 black bears were sampled on both sides of the Trans-Canada highway. The Upper Columbia River region does not appear to be fragmented due to distance either, as 62% of black bears had allele frequencies which were more common in a different quadrant of the study area than the one in which they were sampled.

Future considerations

A mark recapture study must be designed carefully if the goal is to provide population estimates of both brown and black bears. A study whose main goal was to provide a population estimate of a brown bear population may not be appropriate to estimate a sympatric black bear population. A study with brown bears as the primary focus should be placing traps to maximize the probability of capturing brown bears so as to have more captures and re-captures and thereby a better mark recapture estimate. A brown bear study should also maximize the ratio of brown bears to black bears captured to minimize the cost of laboratory analysis. In a study focusing on both brown and black bears there maybe some trapping cells in which there is both good brown bear habitat and good black bear habitat and where placing two traps to maximize the capture of both species is the most appropriate choice. A combined brown and black bear study would ideally have a trap density of at least two traps per female black bear home range during the study. This would allow for a high level of recapture for both bear species and sexes as male black and brown bears as well as female brown bears all have larger home ranges than female black bears.

A study focused on brown bears would have a lower trapping density and lower associated capture rate of black bears, which combined with brown bear focused trap placement would not produce a good black bear population estimate for a number of reasons. The first is that low trapping density can cause closed mark recapture models to fail or have an uncertain bias. If a lot of bears had no chance of being captured as no traps were ever placed in their home ranges there would be a negative bias. This type of negative bias could also result from traps placed to maximized brown bear capture while minimizing black bear captures. A positive bias could result from low trap density if it is possible to have a trap in a bear's home range for only one or a small subset of trapping occasions resulting in a lack of recaptures.

The capture information from the Upper Columbia River region black bear mark recapture study or any non-invasive mark recapture study can be analyzed using the Geographic Information System (GIS). GIS analysis would provide habitat and landscape information on where black bears can or cannot be found and captured. This could provide information on the relative tolerance of black bears to human disturbance and brown bear populations. By analyzing already completed projects more information about the biology and requirements of black bears could be gathered without the need of accompanying fieldwork.

The Upper Columbia River region black bear population has one of the lower levels of black bear density (Table 13). This should be a consideration in the management of this population. The level of harvest should not be the same as for a population in British Columbia or elsewhere with higher black bear densities.

The Upper Columbia River region though it has a relatively low density estimate does have a high effective population size estimate. This raises the question, what areas contribute to immigration into the Upper Columbia River region. The effective population size estimate of 3107 to 15,537 combined with the density estimate, 8.3 black bears per 100 km^2, would indicate that black bears covering an area of 37,434 km^2 to 187,193 km^2 are contributing to the maintenance of the Upper Columbia River region black bear effective population size. This is an area of from 3.9% to 19.7% of the province of British Columbia.

To investigate the possibility of a pattern of genetic structure within a black bear population in British Columbia or elsewhere several things have to be considered. The first consideration is whether all family groups can be identified and dependent cubs removed from the analysis, as dependent offspring will provide a more significant level of structure than will be present after they reach independence. If non-invasive traps are used to acquire genetic tags the traps have to be at a density high enough that family groups are likely to be captured together on multiple occasions. If

microsatellite genotypes are used for the genetic tag enough loci should be included to give a high level of certainty about family relationships. Ideally there would also be a great deal of invasive sampling prior to the mark recapture sampling and/or access to hunter kills post non-invasive sampling in order to provide knowledge about family groups and to provide the age of many sampled individuals. Previous knowledge of family groups and an age framework for samples would enhance the ability to correctly identify cases in which independent related adults are sampled at the same trap from cases where dependent cubs are sampled. A greater number of microsatellite loci would also increase the resolution of the genetic structure.

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